



# The Journal of CLINICAL ENDOCRINOLOGY

Volume 7, 1947

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# CONTENTS OF VOLUME 7

NO. 1, JANUARY, 1947

GONADOTROPIC HORMONE: COMPARISON OF ULTRAFILTRATION AND ALCOHOL-PRECIIPITATION METHODS OF RECOVERY FROM URINE.....	1
<i>E. C. Jungck, W. O. Maddock and C. G. Heller</i>	
THREE UNUSUAL ENDOCRINOPATHIES WITH ASSOCIATED OVARIAN PATHOLOGY: I. OVARIAN AGENESIS. II. PRECOCIOUS PUBERTY. III. VIRILISM.....	11
<i>Minnie B. Goldberg, Alice F. Maxwell and Pearl M. Smith</i>	
PAROXYSMAL HYPERTENSION WITH CONCOMITANT SWELLING OF THE THYROID DUE TO PHEOCHROMOCYTOMA OF THE RIGHT ADRENAL GLAND.....	30
<i>Julius Bauer and Elmer Belt</i>	
THE EFFECT OF THIOURACIL DERIVATIVES ON FETUSES AND INFANTS.....	47
<i>E. Freiesleben and K. Kjerulf-Jensen</i>	
THYROID AND ADRENAL INTERRELATIONS WITH SPECIAL REFERENCE TO HYPOTRICHOSIS AXILLARIS IN THYROTOXICOSIS.....	52
<i>Robert H. Williams</i>	
LETTERS TO THE EDITOR.....	58
ANNOUNCEMENTS.....	65
ASSOCIATION NOTICE.....	68
ABSTRACTS OF CURRENT ENDOCRINE LITERATURE.....	69

NO. 2, FEBRUARY, 1947

EXCRETION OF GLYCOGENIC CORTICOIDS AND OF 17-KETOSTEROIDS IN VARIOUS ENDOCRINE AND OTHER DISORDERS.....	79
<i>Eleanor H. Venning and J. S. L. Browne</i>	
HYPEROPHTHALMOPATHIC SYNDROME IN THYROID DISEASE.....	102
<i>Karl E. Paschkis and A. Cantarow</i>	
VIRILIZING OVARIAN TUMORS.....	115
<i>Jørgen Pedersen</i>	
HYPOGONADOTROPIC EUNUCHOIDISM: REPORT OF CASE WITH FAILURE TO RESPOND TO CHORIONIC GONADOTROPIC HORMONE DUE TO ANTIHORMONES.....	130
<i>Albert Segaloff and William Parson</i>	
ADDISON'S DISEASE FOLLOWED FOR NINE YEARS: CASE REPORT WITH AUTOPSY...	134
<i>Robert C. Moehlig</i>	
CO-EXISTING MYXEDEMA AND HYPERPARATHYROIDISM: CASE REPORT.....	152
<i>Milton Kissin and Hyman Bakst</i>	
THE HYPEREMIA AZT AND THE EVALUATION OF THE HYPEREMIA RAT UNIT OF CHORIONIC GONADOTROPIN.....	159
<i>Bernhard Zondek and Felix Sulman</i>	
PROGRAM OF THE TWENTY-NINTH ANNUAL MEETING.....	165
ANNOUNCEMENT—COUNCIL ON PHARMACY AND CHEMISTRY.....	170

NO. 3, MARCH, 1947

THE CONSTITUTIONAL TYPE OF PRECOCIOUS PUBERTY.....	171
<i>A. M. Hain</i>	
AN EVALUATION OF THE URETHRAL SMEAR AS AN INDEX OF ANDROGENIC DEFICIENCY IN THE MALE.....	186
<i>Eugene J. Cohen</i>	
EFFECTS OF STEROID HORMONES UPON THE DEVELOPMENTAL SEPARATION OF THE PREPUCE FROM THE GLANS PENIS.....	192
<i>L. J. Wells and Curtis J. Lund</i>	
EXCRETION OF 17-KETOSTEROIDS IN ANKYLOSING SPONDYLARTHRITIS AND IN RHEUMATOID ARTHRITIS: A PRELIMINARY REPORT.....	201
<i>Roland A. Davison, Peter Koets and William C. Knzell</i>	
INSULIN REGULATION IN ONE HUNDRED AND TWENTY-SIX DIABETIC CHILDREN...	205
<i>Herman O. Mosenthal and Albert P. Rosen</i>	
THYROTOXICOSIS COMPLICATED BY SEVERE IODISM: PREPARATION FOR SURGERY WITH PROPYL-THIOURACIL.....	212
<i>Edward A. Newman and Philip H. Ross</i>	
LETTER TO THE EDITOR.....	219
ASSOCIATION NOTICE.....	222
ANNOUNCEMENT OF AWARDS.....	223
ABSTRACTS OF CURRENT ENDOCRINE LITERATURE.....	225

NO. 4, APRIL, 1947

RADIO IODINE: ITS USE AS A TOOL IN THE STUDY OF THYROID PHYSIOLOGY.....	235
<i>Rulon W. Rawson and Janet W. McArthur</i>	
THE EFFECT OF TRAUMA AND DISEASE ON THE URINARY 17-KETOSTEROID EXCRETION IN MAN.....	264
<i>Anne P. Forbes, Elizabeth C. Donaldson, Edward C. Reifenstein, Jr. and Fuller Albright</i>	
THE DIAGNOSIS OF HYDATIDIFORM MOLE BY GONADOTROPIC HORMONE ASSAY USING THE SOUTH AFRICAN FROG, <i>Xenopus Laevis</i> .....	289
<i>Abner I. Weisman and Christopher W. Coates</i>	
MALE HYPOGONADISM TREATED BY SUBLINGUAL METHYLTESTOSTERONE.....	293
<i>Rita S. Finkler</i>	
ABSTRACTS OF CURRENT ENDOCRINE LITERATURE.....	303

NO. 5, MAY, 1947

POLYOSTOTIC FIBROUS DYSPLASIA: A DEFENSE OF THE ENTITY.....	307
<i>Fuller Albright</i>	
THE ACTIVITY OF ARGINASE IN RED BLOOD CELLS.....	325
<i>Genevieve C. Covolo and Randolph West</i>	

THE EXCRETION OF 11-OXYCORTICOSTEROID-LIKE SUBSTANCES BY NORMAL AND ABNORMAL SUBJECTS.....	331
<i>Nathan B. Talbot, Fuller Albright, Annette H. Saltzman, Aniela Zygmuntowicz and Robert Wirom</i>	
THE PREGNANDIOL PRECIPITATION TEST—CLINICAL APPLICATION OF A RAPID METHOD FOR THE DIAGNOSIS OF PREGNANCY.....	351
<i>Harold C. Mack and Arthur E. Parks</i>	
THE METABOLISM OF SINGLE THERAPEUTIC DOSES OF THE NATURAL ESTROGENS IN HUMAN SUBJECTS.....	364
<i>Benjamin F. Stimmel</i>	
PROGRAM—LAURENTIAN HORMONE CONFERENCE.....	374
ABSTRACTS OF CURRENT ENDOCRINE LITERATURE.....	376

---

 NO. 6, JUNE, 1947

SYNDROME OF RUDIMENTARY OVARIES WITH ESTROGENIC INSUFFICIENCY AND INCREASE IN GONADOTROPINS.....	385
<i>E. B. del Castillo, F. A. de la Balze and J. Argonz</i>	
TREATMENT OF CARCINOMA OF THE HUMAN BREAST WITH TESTOSTERONE PROPIONATE.....	423
<i>Howard Schicander and Horace N. Marvin</i>	
SYMMETRIC CEREBRAL CALCIFICATION WHICH FOLLOWED POSTOPERATIVE PARATHYROID INSUFFICIENCY: REPORT OF A CASE.....	433
<i>Irrin S. Siglin, L. M. Eaton, John D. Camp and Samuel F. Haines</i>	
FEMINIZING TUMOR OF THE TESTIS—PRESUMABLY ABERRANT ADRENOCORTICAL TUMOR.....	438
<i>Erling Østergaard</i>	
ABSTRACTS OF PAPERS READ AT THE TWENTY-NINTH ANNUAL MEETING.....	446
ABSTRACTS OF CURRENT ENDOCRINE LITERATURE.....	471

---

 NO. 7, JULY, 1947

BILATERAL FAMILIAL PHAEOCHROMOCYTOMATA WITH PAROXYSMAL HYPERTENSION: SUCCESSFUL SURGICAL REMOVAL OF TUMORS IN TWO CASES, WITH DISCUSSION OF CERTAIN DIAGNOSTIC PROCEDURES AND PHYSIOLOGICAL CONSIDERATIONS.....	475
<i>Evan Calkins and John Eager Howard</i>	
SYNDROME PRODUCED BY ABSENCE OF THE GERMINAL EPITHELIUM WITHOUT IMPAIRMENT OF THE SERTOLI OR LEYDIG CELLS.....	493
<i>E. B. del Castillo, Armando Trabucco and F. A. de la Balze</i>	
URINARY EXCRETION OF 17-KETOSTEROIDS IN VARIOUS CONDITIONS OF OLIGOPHRENIA CORRELATED WITH SOME AUTOPSY OBSERVATIONS.....	503
<i>Clemens E. Benda and Emily May Birby</i>	
ESTRONE CLEARANCE TEST IN INFECTIOUS HEPATITIS.....	519
<i>Bernhard Zondek and Riwka Black</i>	

ANNOUNCEMENT OF THE 1948 MEETING OF THE ASSOCIATION.....	530
ABSTRACTS OF CURRENT ENDOCRINE LITERATURE.....	531
<hr/>	
NO. 8, AUGUST, 1947	
SERUM GLUCURONIDASE ACTIVITY DURING NORMAL AND TOXEMIC PREGNANCY... <i>Donald F. McDonald and Lester D. Odell</i>	535
RELATION OF URINARY STEROIDS TO THE DIAGNOSIS OF ADRENAL CORTICAL TUMORS AND ADRENAL CORTICAL HYPERPLASIA: QUANTITATIVE AND ISOLATION STUDIES..... <i>Edwin J. Kepler and Harold L. Mason</i>	543
PLASMA PROTEIN PATTERN (TISELIUS ELECTROPHORETIC TECHNIQUE) IN CUSHING'S SYNDROME..... <i>Lena A. Lewis and E. Perry McCullagh</i>	559
THE CO-EXISTENCE OF HYPERTHYROIDISM AND PREPUBERAL EUNUCHOIDISM IN A MALE..... <i>Joseph Ballinger</i>	566
USE OF MASSIVE DOSES OF VITAMIN A IN THE TREATMENT OF HYPERTHYROIDISM. A PRELIMINARY REPORT..... <i>Samuel Simkins</i>	574
GYNECOMASTIA..... <i>Willerd H. Spankus and Robert S. Grant</i>	586
ANNOUNCEMENT OF THE 1948 MEETING OF THE ASSOCIATION FOR THE STUDY OF INTERNAL SECRETIONS.....	602
ANNOUNCEMENT OF GOITER SOCIETY AWARD.....	603
ABSTRACTS OF CURRENT ENDOCRINE LITERATURE.....	604
<hr/>	
NO. 9, SEPTEMBER, 1947	
THE FACTOR OF PREVIOUS TREATMENT IN EXPERIMENTAL MENSTRUATION..... <i>Doris H. Phelps</i>	611
THE CLINICAL SIGNIFICANCE OF HYPEROSTOSIS FRONTALIS INTERNA..... <i>Norman G. Schneeberg, George Woolhandler and Rachmiel Levine</i>	624
ACROMEGALY ASSOCIATED WITH AMYOTROPHIC LATERAL SCLEROSIS AND ACROMEGALY OF THE AMYOTROPHIC TYPE..... <i>E. Perry McCullagh and J. S. Hewlett</i>	636
CONJUNCTIVAL AND CORNEAL LESIONS IN HYPERCALCEMIA..... <i>Frank B. Walsh and John Eager Howard</i>	644
PREGNANCY TEST USING THE MALE TOAD..... <i>Carlos Galli Mainini</i>	653
LETTER TO THE EDITOR.....	659

BOOK REVIEW.....	661
ANNOUNCEMENT OF THE 1948 MEETING OF THE ASSOCIATION.....	662
ANNOUNCEMENT OF AWARDS AND FELLOWSHIP OF THE ASSOCIATION.....	663
ANNOUNCEMENT OF GOITER SOCIETY AWARD.....	664

---

 NO. 10, OCTOBER, 1947

THE SYNDROME OF CONGENITALLY APLASTIC OVARIES WITH SEXUAL INFANTILISM, HIGH URINARY GONADOTROPINS, SHORT STATURE AND OTHER CONGENITAL ABNORMALITIES. TABULAR PRESENTATION OF TWENTY-FIVE PREVIOUSLY UNPUBLISHED CASES.....	665
<i>H. Lissner, L. E. Curtis, R. F. Escamilla and Minnie B. Goldberg</i>	
A CLINICAL EVALUATION OF DIENESTROL, A SYNTHETIC ESTROGEN.....	688
<i>A. E. Rakoff, K. E. Paschkis and A. Cantarow</i>	
A SIMPLE QUANTITATIVE COLORIMETRIC METHOD FOR ESTROGENIC STEROIDS....	701
<i>Herman Cohen and Robert W. Bates</i>	
EXPERIMENTAL USE OF TESTOSTERONE COMPOUNDS IN PREMATURE INFANTS.....	708
<i>E. Kost Shelton, Arthur E. Varden and Jerome S. Mark</i>	
GOITER ON AN IODINE-FREE DIET GROWN BY HYDROPONICS AND EXCLUDING ANY GOITER NOXA.....	714
<i>J. F. McClendon and W. C. Foster</i>	
PHEOCHROMOCYTOMA WITH DIABETES .....	716
<i>Martin G. Goldner</i>	
ANNOUNCEMENT OF THE 1948 MEETING OF THE ASSOCIATION .....	724
ANNOUNCEMENT OF AWARDS AND FELLOWSHIP OF THE ASSOCIATION.....	725
ANNOUNCEMENT OF GOITER SOCIETY AWARD.....	726
ABSTRACTS OF CURRENT ENDOCRINE LITERATURE.....	727

---

 NO. 11, NOVEMBER, 1947

EFFECT OF TESTOSTERONE ON THE EXCRETION OF GLYCOGENIC CORTICOIDS.....	729
<i>Eleanor H. Venning and J. S. L. Browne</i>	
TRUE HERMAPHRODITISM. ENDOCRINE STUDIES IN A CASE OF OVOTESTIS .....	741
<i>John C. Weed, Albert Segaloff, W. B. Wiener and J. W. Douglas</i>	
A COMPARATIVE STUDY OF VAGINAL AND CERVICAL CORNIFICATION IN HUMAN SUBJECTS.....	749
<i>J. E. Ayre, P. M. Chevalier and W. B. Ayre</i>	
THE USE OF HYPERTONIC SALINE INFUSIONS IN THE DIFFERENTIAL DIAGNOSIS OF DIABETES INSIPIDUS AND PSYCHOGENIC POLYDIPSIA.....	753
<i>Anne C. Carter and Jacob Robbins</i>	

THIOURACIL IN THE TREATMENT OF HYPERTHYROIDISM COMPLICATING PREGNANCY AND ITS EFFECT ON THE HUMAN FETAL THYROID. ....	767
<i>M. James Whitclaw</i>	
ANNOUNCEMENT OF THE 1948 MEETING OF THE ASSOCIATION. ....	774
ANNOUNCEMENT OF AWARDS AND FELLOWSHIP OF THE ASSOCIATION. ....	775
POSTGRADUATE COURSE IN ENDOCRINOLOGY. ....	777
ANNOUNCEMENT OF GOITER SOCIETY AWARD. ....	778
ABSTRACTS OF CURRENT ENDOCRINE LITERATURE. ....	779

## NO. 12, DECEMBER, 1947

SPERMATOGENESIS IN A "PAN-HYPOPHYTARY" EUNUCHOID, AS THE RESULT OF TESTOSTERONE THERAPY. ....	781
<i>Laurance W. Kinsell</i>	
THE ENDOCRINE DISORDERS ASSOCIATED WITH CUSHING'S SYNDROME AND VIRIL- ISM. ....	787
<i>A. C. Crooke</i>	
A RAPID METHOD FOR THE DETERMINATION OF TOTAL URINARY 17-KETOSTER- OIDS. ....	795
<i>I. J. Drechter, S. Pearson, E. Bartczak and T. H. McGavack</i>	
SPECIFIC RENAL FUNCTIONS IN HYPERTHYROIDISM AND MYXEDEMA. ....	801
<i>A. C. Coreoran and Irvine H. Page</i>	
REPORT OF A CASE SHOWING CONGENITAL DEFECTS, SHORT STATURE, RETARDED SEXUAL DEVELOPMENT AND NO URINARY GONADOTROPINS. ....	897
<i>George B. Dorff, David H. Appelman and Arthur Liverson</i>	
THE TREATMENT OF THYROTOXICOSIS WITH AMINOTHIAZOLE—RESULTS IN TWENTY-THREE CASES. ....	812
<i>Jeannette S. McConnell, John W. Frost, Robert W. Wilbur and Edward Rose</i>	
IODINE-LACK THEORY AND ENDEMIC GOITER. ....	820
<i>H. Ucko</i>	
LETTERS TO THE EDITOR. ....	828
ANNOUNCEMENT OF THE 1948 MEETING OF THE ASSOCIATION. ....	831
ANNOUNCEMENT OF AWARDS AND FELLOWSHIP OF THE ASSOCIATION. ....	832
ANNOUNCEMENT OF GOITER SOCIETY AWARD. ....	833
POSTGRADUATE COURSE IN ENDOCRINOLOGY. ....	834
AUTHOR INDEX TO VOLUME 7. ....	835
SUBJECT INDEX TO VOLUME 7. ....	845

# The Journal of CLINICAL ENDOCRINOLOGY

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## GONADOTROPIC HORMONE: COMPARISON OF ULTRAFILTRATION AND ALCOHOL-PRE- CIPITATION METHODS OF RECOVERY FROM URINE<sup>1</sup>

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**T**REATMENT of the hypogonadal patient, either male or female, cannot reasonably be instituted until a definitive diagnosis is established. The definitive diagnosis must state whether gonadal failure primarily involves the gonad or is secondary to failure of pituitary gonadotropin secretion. The only sure way to make this differentiation is to determine the amount of gonadotropic hormone in blood or urine.

If gonadal failure is secondary to pituitary failure, urinary gonadotropins will be distinctly lower than normal and stimulation by administration of gonadotropins is indicated. If gonadal failure is primary in the gonad, urinary gonadotropins will be distinctly higher than normal and administration of gonadotropins will fail to stimulate the gonad. Therefore substitutional therapy with sex hormones is permissible.

As yet methods for recovering and assaying urinary gonadotropins have been too complex, time-consuming and expensive for clinic and hospital laboratories, and as a consequence have been limited largely to research

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<sup>2</sup> Schering Fellow in Endocrinology.



laboratories. Recently Gorbman (1) introduced a new principle for recovering urinary hypophyseal hormones by use of the ultrafilter, which appeared to offer the possibility of overcoming these difficulties. Therefore we compared ultrafiltration with the alcohol-precipitation-dialysis method of Heller and Chandler (4).

The two methods will be compared from the point of view of (1) complexity, (2) time consumed, (3) expense, and (4) quantitative recovery. Both methods were conducted as originally described (1 and 2). Twelve-hour overnight urine specimens were collected from patients presumed to have lower than normal, normal, and higher than normal titers of urinary gonadotropins. Urines from each patient were pooled, and equal amounts of the pooled urine concentrated by each of the two methods concurrently. The concentrates were assayed on 22-24 day old female Sprague Dawley rats, injecting once every twelve hours for six injections. Twenty-four hours after the last injection the rats were sacrificed and the uterine and ovarian weights used as the assay end-point.

(1) Complexity of the two methods may be compared from the following résumé of each:

In *ultrafiltration*, the urine is placed in a pressure filter and forced through a collodion membrane. When filtration is completed, the membrane, along with the protein hormone residue which has not passed through the pores of the membrane due to the large size of the protein molecule, is removed from the filter and placed in alcohol-ether solution in a centrifuge tube. The alcohol-ether dissolves the collodion membrane and at the same time precipitates the protein hormone. The solution is centrifuged, the supernatant poured off, and the residue repeatedly washed with alcohol-ether. After the last centrifugation, the residue is allowed to dry in the centrifuge tube. At the time of assay the dry precipitate is extracted with water.

In *alcohol precipitation*, the urine is precipitated with five volumes of alcohol; the supernatant liquid is decanted and the precipitate transferred to a centrifuge tube where it is centrifuged, washed with ether, and dried. The precipitate is suspended in water, transferred to a cellophane bag and dialyzed against running tap water. After dialysis the hormone-containing suspension is transferred to a centrifuge tube, centrifuged, and the precipitate discarded. The clear supernatant solution is transferred to a beaker where it is reprecipitated with five volumes of alcohol. This precipitate is transferred to a centrifuge tube, centrifuged, washed with ether, and dried. When desired for assay, the dried precipitate is extracted with water.

It is apparent that the alcohol-precipitation method requires more

materials and more manipulation than does ultrafiltration. With each manipulation or transfer from vessel to vessel, possibilities of loss or error are increased.

(2) Time consumption is compared in the following tabulation:

<i>Ultrafiltration:</i>	<i>Time-Hours</i>
a. Collect protein on collodion membrane by ultrafiltration	3
b. Dissolve membrane in alcohol-ether (protein is precipitated) and	1
c. Wash 5 times with alcohol-ether	
d. Extract with 6 cc. H <sub>2</sub> O	1
Total	5
<i>Alcohol-precipitation:</i>	
a. Precipitate with 5 volumes alcohol	12-24
b. Decant, wash precipitate with ether, dry	5
c. Suspend in H <sub>2</sub> O, dialyze	12
d. Centrifuge, reprecipitate supernatant with 5 volumes alcohol	12-48
e. Decant, wash precipitate with ether, dry	2
f. Extract in 6 cc. H <sub>2</sub> O	1
Total	44-92

(3) Expense. The main items of expense with the *alcohol-precipitation* method are labor and alcohol. *Ultrafiltration* reduces the labor and eliminates the use of large volumes of alcohol.

(4) Quantitative recovery. Comparisons were made by conducting assays on urines from patients presenting a variety of syndromes. These are discussed separately below. The syndromes involved primarily the gonads in some instances, and primarily the pituitary in others. Both male and female subjects were chosen. There is reason to suspect that the ratio of follicle-stimulating hormone to interstitial-cell-stimulating hormone (ICSH, also called luteinizing hormone) is not constant from syndrome to syndrome. For example, if both methods recover equal amounts of FSH but unequal amounts of ICSH, then in a syndrome in which predominately FSH is excreted the two methods would recover approximately equal amounts of gonadotropic substances. If, on the other hand, urine containing predominately ICSH is analyzed, one method might recover much more gonadotropic substance than the other. Therefore it seemed important to include examples of as many syndromes as possible. A second reason for listing the results of the gonadotropin assays by syndromes is to illustrate the clear-cut differences that can be drawn between normal, hypoexcretion and hyperexcretion types.

## NORMAL GONADOTROPIN EXCRETION

Normal men. Urines from nine men were compared, using six-hour and twelve-hour aliquots. Fifty separate assays were conducted. At the six-hour level, rat ovarian weights were indistinguishable from those of the uninjected controls. However, uterine weights were increased by using concentrates from each method. At the twelve-hour level, the alcohol precipitation and ultrafiltration methods elicited equal ovarian responses

TABLE 1. NORMAL GONADOTROPIN EXCRETION

Type of Subject	No. of Cases	Urine: Aliquot Hours	No. of Assays	Uterine Weight <sup>1</sup>		Ovarian Weight	
				Method		Method	
				Ultrafiltration	Alcohol-precipitation	Ultrafiltration	Alcohol-precipitation
Normal Males	9	6	15	mg. 84	mg. 63	mg. 9.4	mg. 10.2
		12	35	99	89	22.9	20.7
Endocrinopathies	7	6	14	41	47	10.8	11.1
		12	19	53	100	13.5	19.4
Total	16	6	29	60	57	10.2	10.5
		12	54	81	92	19.0	20.3

Uninjected control rats<sup>2</sup> 62 Range: 17.2 to 60.3 Ave. 31.0 Range: 3.9 to 19.0 Ave. 10.7

<sup>1</sup> Uterine weights are recorded after fluid has been expressed.

<sup>2</sup> Uninjected control rats were included with each series of assays conducted and serve as controls for Tables 2, 3, and 4 as well.

of approximately 20 mg., along with maximal uterine stimulation (Table 1). Thus one may conclude that the methods recover equal amounts of gonadotropic hormone from urine of normal men, and that each method is capable of detecting the hormone in six-hour aliquots.

Endocrinopathies associated with normal gonadotropin excretion. These included one case each of Addison's disease, Cushing's syndrome, adrenogenital syndrome, pseudohermaphrodisism having bilateral ovo-

testes, myxedema, simple hirsutism, and pituitary gigantism. Thirty-three separate assays were performed.

The concentrates of the two methods were apparently of identical potency at the six-hour level, but assaying at the twelve-hour level revealed that alcohol-precipitation had recovered slightly greater amounts. The uterine response to six-hour aliquots was positive in some patients and negative in others, but in no instance did ovarian weight response occur.

The difference in ovarian response at the twelve-hour level seen in Table 1 is not as great as the figures seem to indicate, since several ovaries of less than normal weight were included in compiling the ultrafiltration data. A reduction in ovarian weight often follows administration of small amounts of gonadotropin, if the dose is sufficiently large to cause stimulation of ovarian function without causing an increase in size (3). The ovarian stimulation is reflected by the uterine weight increase.

Averaging the assays of normal men with the assays of the various endocrinopathies exhibiting normal gonadotropin levels, it is readily seen that the ultrafiltration method and the alcohol-precipitation method are equally effective in detecting normal gonadotropin titers in urine, in both six-hour and twelve-hour urine aliquots.

#### INCREASED GONADOTROPIN EXCRETION IN HYPOGONADAL SYNDROMES

Examples of several of the hypogonadal syndromes which are characterized by hypergonadotropic excretion were chosen for comparing the two methods.

1. **Menopause.** The urines of three women over forty years of age were studied by both methods and the elevated titers confirmed the diagnosis of menopause.

2. **Premature menopause.** In a woman, age 26 years, with a history of abrupt amenorrhea accompanied by menopausal symptoms, the diagnosis of premature menopause was confirmed by finding elevated urinary gonadotropin titers.

3. **Ovarian agenesis.** Four cases of ovarian agenesis were studied by the two methods, and each method revealed the high urinary gonadotropin titer present in this syndrome.

4. **Klinefelter's syndrome** (*prepuberal or puberal seminiferous tubule failure*).

The three cases of Klinefelter's syndrome studied by both methods showed uniformly high titers of gonadotropin excretion.

5. **Adult seminiferous tubule failure.** Three cases of adult seminiferous tubule failure revealed very high urinary gonadotropin levels by both ultra-

filtration and alcohol-precipitation. This confirmed the information obtained by testicular biopsy that the testis was primarily involved.

6. **Male climacteric.** The one case of male climacteric compared by the two methods showed distinctly increased urinary gonadotropin titers by each method, coincident with the symptoms characteristic of this syndrome.

The recovery of gonadotropins in the above-mentioned hypergonadotropic syndromes are compared in Table 2. A significantly higher recovery in the one, two and three-hour aliquots was obtained when concentration was performed by alcohol-precipitation. Less striking differences were

TABLE 2. INCREASED GONADOTROPIN EXCRETION

Type of Subject	No. of Cases	Urine: Aliquot Hours	No. of Assays	Uterine Weight		Ovarian Weight	
				Method		Method	
				Ultrafiltration	Alcohol-precipitation	Ultrafiltration	Alcohol-precipitation
Hypogonadal syndromes	15	1	9	mg. 47	mg. 64	mg. 16.5	mg. 24.7
		2	9	77	101	17.8	45.1
		3	22	113	114	38.2	63.6
		6	32	103	100	60.0	62.6
		12	30	110	119	75.6	86.5

noted at the six-hour and twelve-hour levels. Maximal uterine weight response was attained at the three, six and twelve-hour dose levels by both methods.

It must be concluded that in hypergonadotropic syndromes under the conditions of the assay comparison, alcohol-precipitation consistently recovers a greater amount of gonadotropin than ultrafiltration. This difference may be explained by our recent observation that in the ultrafiltration process the pH of the urine directly affects the amounts recovered, whereas variations in pH do not materially affect the amounts recovered by alcohol-precipitation. Present indications are that the urinary pH must be 6.0 or lower for effective recovery of gonadotropins by ultrafiltration. The pH of some of the urines may have been above this figure and therefore caused loss of the active principle.

The important point illustrated by this table is that in the hypergonadotropic hypogonadal syndromes both ultrafiltration and alcohol-precipitation recover many times the amount of gonadotropic hormone found in normal urines. They therefore provide a means of differentiating elevated excretion of gonadotropic hormone from normal excretion.

#### DECREASED GONADOTROPIN EXCRETION IN HYPOGONADAL SYNDROMES

Examples of several of the hypogonadal syndromes which are characterized by hypogonadotropic excretion were chosen for comparing the two methods.

Prepuberal children are by definition hypogonadal until puberty is attained, and it is well known that until then gonadotropin excretion is negligible in amount. Five such children were chosen for comparison with normal adults.

TABLE 3. DECREASED GONADOTROPIN EXCRETION

Type	No. of Cases	Urine: Aliquot Hours	No. of Assays	Uterine Weight		Ovarian Weight	
				Method		Method	
				Ultrafiltration	Alcohol-precipitation	Ultrafiltration	Alcohol-precipitation
Hypogonadal syndromes	10	6	12	31.7	29.6	12.0	10.7
		12	13	17.6	30.9	10.4	11.1

**Hypogonadotropic Eunuchoidism.** One example of this syndrome was studied.

**Anorexia nervosa.** Two cases, one of each sex, were studied.

Artificial suppression of gonadotropin excretion by the administration of 5 mg. of diethylstilbesterol daily was performed in two cases. One with ovarian agenesis had elevated titers before treatment; the other, a pseudohermaphrodite, had normal titers.

In the 25 assays conducted at the six and twelve-hour levels no hormone was detected, irrespective of the method of concentration. Thus both methods are capable of distinguishing between hypogonadotropic hypogonadism and normal gonadotropic excretion.

#### ACROMEGALY

Three cases of acromegaly which were studied proved interesting in that one example each of decreased, normal, and increased gonadotropin ex-

cretion was discovered. In the patients with decreased and increased excretion, the methods agreed closely. In the third patient, alcohol-precipitation recovered hormone which was within the variation of the normal range, but the single test performed at the twelve-hour level by ultrafiltration detected none.

### DISCUSSION

The data summarized in Figure 1 show that either method will sharply delineate the hypogonadotropic syndromes from normal, or syndromes having normal gonadotropin excretion. In turn, the hypergonadotropic syndromes can be sharply delineated from the other syndromes.

TABLE 4. ACROMEGALY

Type	No. of Cases	Urine: Aliquot Hours	No. of Assays	Uterine Weight		Ovarian Weight	
				Method		Method	
				Ultrafiltration	Alcohol-precipitation	Ultrafiltration	Alcohol-precipitation
Decreased	1	6	4	41	29	12.9	11.4
		12	2	19	18	9.4	8.4
Normal	1	12	3	40	56	12.0	12.0
Increased	1	6	4	86	116	75.4	88.4
		12	2	93	121	108.5	56.3

**Terminology.** The terms "gonadotropic hormone" and "follicle-stimulating hormone" have been used interchangeably to signify the material in human urine recovered by the various concentration methods. The term "gonadotropic hormone" actually includes all the factors (of which follicle-stimulating hormone is one) which stimulate the function or morphologic development of the gonads. Greep et al. (2), found that purified follicle-stimulating hormone produces ovarian morphologic development but does not cause the secretion of estrogen. The addition of interstitial-cell-stimulating hormone is necessary to produce the secretion of estrogen. In the light of these observations, when uterine weight stimulation is used as an end-point it then follows that both factors are present. Therefore, the term "follicle-stimulating hormone" is incomplete, whereas the term "gonadotropic hormone" satisfies the requirements.

**Assay end-point.** We have used both uterine and ovarian weights as assay end-points. At lower dose levels (six-hour urine aliquot of a normal person), uterine weight increase occurs without ovarian weight increase. At higher dose levels, ovarian weight increase, along with maximal uterine weight increase, is observed. Thus, by using both uterine and ovarian weights as assay end-points, the range of usefulness of the assay animal is increased. Instead of using arbitrary units, results are expressed as

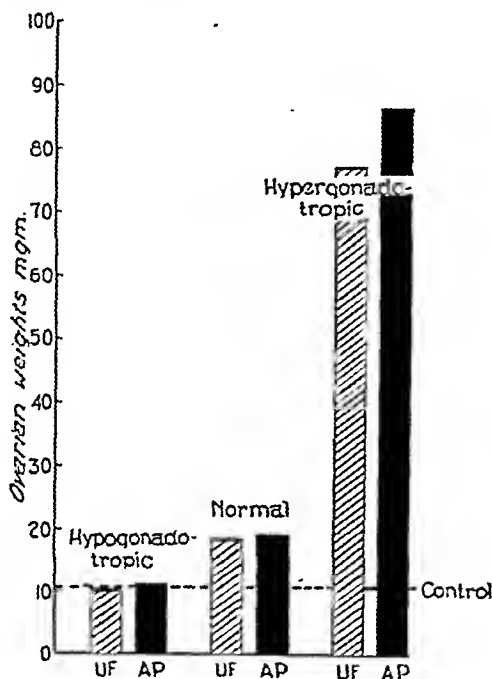


FIG. 1. Comparison of recovery of urinary gonadotropins by ultrafiltration (UF) and alcohol-precipitation (AP). Comparisons are compiled from the assays of twelve-hour urine specimens included in Tables 1, 2, and 3. The dotted line labelled "Control" is the average ovarian weight of uninjected control rats.

actual uterine and ovarian weights. This facilitates comparison and interpretation of the data by others.

When minimal or fixed organ weights (uterine or ovarian) are used as the end-point, and results described in terms of units, the organ weight becomes the constant, and the dosage of hormone or urine aliquot becomes the variable. This means that many dose levels and hence many assay animals must be used to arrive at the exact dose level which will elicit the arbitrarily chosen end-point. In contrast, by using the dose as the constant, and organ weight response as the variable, each assay animal yields definite information. In addition to the quantitative response,



qualitative information may be obtained by observing development of ovarian follicles, blood points, corpora lutea, and the stage of uterine development, estrogenic or progestational.

### SUMMARY

Since gonadotropin determinations occupy a pivotal position in arriving at a definitive diagnosis in a variety of endocrine diseases, it is important to have a method which is adaptable to the average clinic or hospital laboratory. Therefore the newly introduced technic of ultrafiltration was compared with the well-established technic of alcohol-precipitation. Comparisons were made on urines from normal men, menopausal women, cases of anorexia nervosa, Klinefelter's syndrome, ovarian agenesis, hypogonadotropic eunuchoidism, male climacteric, Addison's disease, acromegaly, myxedema, Cushing's syndrome, and pseudohermaphroditism, as well as other endocrinopathies.

Ultrafiltration proved to have great advantage over alcohol-precipitation in that it was (1) less complex, therefore easily carried out by the average technician, reducing the possibility of errors, (2) less time-consuming (5 versus 44-92 hours), (3) less expensive, and (4) recovered almost as much hormone quantitatively as the alcohol-precipitation method.

By assaying for gonadotropic hormone using the ultrafiltration technic, one can readily distinguish between the hypergonadotropic and hypogonadotropic syndromes, and normal.

### ACKNOWLEDGMENT

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# THREE UNUSUAL ENDOCRINOPATHIES WITH ASSOCIATED OVARIAN PATHOLOGY: I. OVARIAN AGENESIS. II. PRECOCIOUS PUBERTY. III. VIRILISM<sup>1</sup>

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## INTRODUCTION

THE ORIGINAL intent of the authors in grouping the following three dissimilar cases under one title was to emphasize the nonneoplastic ovarian origin of the endocrinopathies. It soon became apparent that the primary ovarian origin of the syndromes could not be upheld unequivocally. Nevertheless, inasmuch as the three cases present such a dramatic contrast, each to the other, it was thought appropriate to leave them as a triad: the first, an instance of congenital ovarian aplasia; the second, precocious hyperactivity of the ovarian function; and the third, a pathologic perversion of the same function. In all three the diagnosis was verified by surgical exploration, and in each instance benefit was obtained by appropriate therapy.

## I. OVARIAN AGENESIS

In March 1942, Varney, Kenyon and Koch (31) published an article entitled, "Association of short stature, retarded sexual development and high urinary gonadotrophin titers in women; ovarian dwarfism." When they pointed out the association of either absent or at most rudimentary ovaries with this picture, they had introduced a new syndrome to the medical world. Thus was explained the mystery of the complete lack of response to pituitary gonadotropic hormones of many cases hitherto erroneously diagnosed as hypophyseal infantilism. In November of the same year, Albright, Smith and Fraser (1) further elucidated the same syndrome and carefully differentiated it from pituitary infantilism, or panhypopituitarism, as they choose to call it. Wilkins and Fleischmann (33), in August 1944, published a masterly exposition of the subject and gave the new syndrome its name, "ovarian agenesis." They analyzed and summarized the findings in the 47 cases thus far recorded in the literature,\*

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\* The 7 cases mentioned by Shereshevski (24) were not included in this tabulation.

of which 18 had been verified either by surgical exploration, peritoneoscopy, or autopsy.

As with all new syndromes, the next few years should see many more cases added to the series. The one which we are about to report represents the youngest thus far to be diagnosed and proved.

#### CASE RECORD

*L.L.* (private patient, M.B.G.) was first seen in 1941 at the age of eight years because of failure to grow in height. All of her family were normal except for a maternal uncle who did not grow in height or mature sexually until his second year at college.

The patient was a full term baby and weighed 6 pounds at birth. She had 2 teeth before she was 4 months of age, and 16 teeth before she was 1 year of age. She had always been tiny and had been growing at an average rate of 1 inch per year. Otherwise her past history was not remarkable. Her performance at school had been average.

In appearance, this 8 year old girl had the facies and dimensions of a 5 year old (Fig. 1a). Her measurements at this time were as follows:

Height.....	42½ inches
Weight.....	45½ pounds
Span.....	41½ inches
Upper segment (pubis to vertex).....	23½ inches
Lower segment (pubis to floor).....	19½ inches
Ratio of upper to lower.....	1.18
Circumference—neck.....	11 inches
Circumference—chest.....	24½ inches
Circumference—waist.....	23 inches
Circumference—abdomen.....	23½ inches

Her skeletal build was somewhat disproportionate in that the lower legs were quite short and unusually muscular. There were 12 permanent teeth present. All the teeth were small, the upper incisors seeming to incline lingually. The gingivae were unusually heavy. Otherwise the physical findings were not unusual. The osseous development was only slightly retarded, being estimated at between six and seven years. The roentgenograms of the skull were normal except for a slight underdevelopment of the clinoid processes. On ophthalmologic examination, the fundi were normal, as were the visual fields.

**Laboratory data:** The urine and blood were normal. The whole blood cholesterol was 137 mg. %. A basal metabolic rate was not attempted.

The diagnosis at this time was pituitary infantilism. The child was observed closely over a period of five years, during which her only regular therapy was adequate diet, vitamins, and desiccated thyroid in doses averaging .045 gm. daily, which seemed to be her tolerance dose. For a short period she was given oral enteric-coated pituitary substance, without noticeable benefit. Skull plates, bone age studies, and ophthalmologic examinations were repeated at regular intervals. The record of her growth can be seen on Chart I.

From July to September 1942, a trial of stilbestrol therapy was made. During these two months she grew ⅓ of an inch in height, began to show breast development and to grow axillary and pubic hair. This therapy was promptly discontinued because of her age (10½ years), and all signs of beginning maturity promptly regressed.



FIG. 1a. Age 8 years; height 42½ inches.

FIG. 1b. Age 12½ years.

Figure 1b represents the patient at the age of 12½ years. At this time she was a short, plump, alert, intelligent girl with the following measurements:

Height.....	49½ inches
Weight.....	67½ pounds
Span.....	51 inches
Upper segment (pubis to vertex).....	25½ inches
Lower segment (pubis to floor).....	24 inches
Ratio of upper to lower.....	1.07
Circumference—chest, at nipple line.....	27½ inches

The span was relatively long for her height, whereas the lower extremities were short in proportion. The trunk was of ebildish configuration. There was no webbing of the neck or increased carrying angle of the arms (Turner's (30) syndrome). The hands and fingers were not remarkable. The musculature was normally developed. The subcutaneous fat was somewhat increased in amount, but uniformly distributed. The skin was of delicate texture, that of the lower extremities showing considerable purplish mottling.

There was no unusual pigmentation. The teeth were now quite normal due to orthodontic care, but the gingivae were still hypertrophic. The sexual development was completely infantile. The labia were underdeveloped and the clitoris was not enlarged. On rectal examination, a tiny cordlike structure was palpated in the midline. This probably represented the uterus and cervix. The adnexa were not felt. The breasts were infantile and there was a total absence of sexual hair. The osseous development, however, had kept

## OVARIAN AGENESIS

L.L. GROWTH CHART

DATE OF BIRTH DEC. 3, 1932

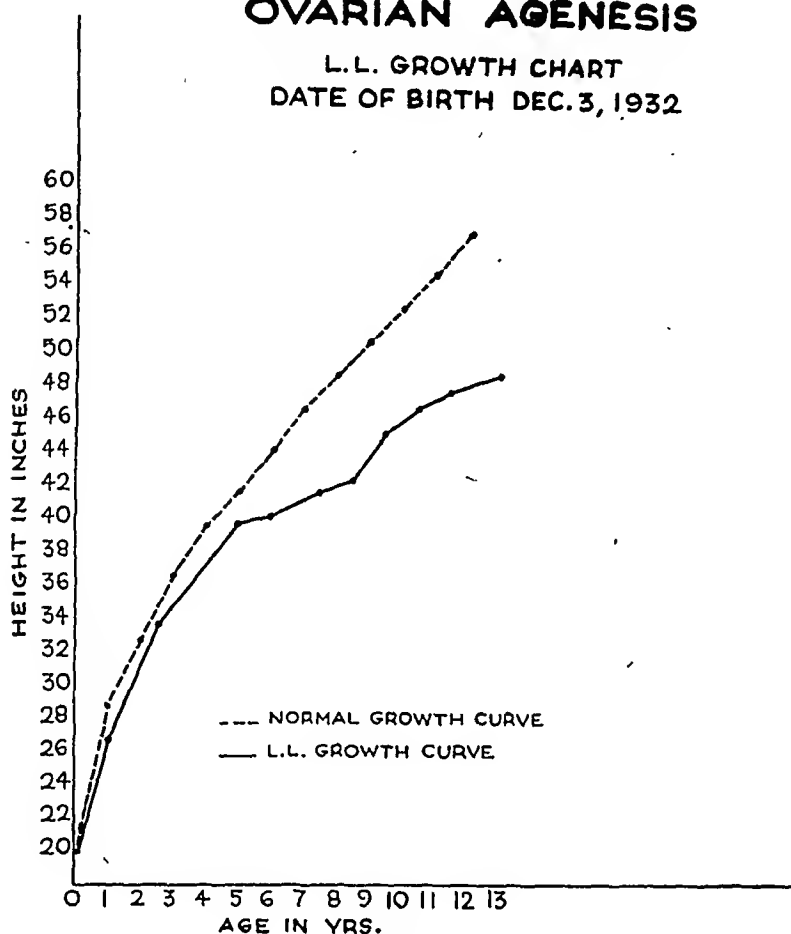


CHART I

pace with the passage of time, so that at the age of twelve and one-half years the bone age was estimated at between eleven and twelve years. The epiphysis for the external condyle of the humerus had begun to develop. The epiphyses for the trochlea and capitulum were ununited, as were those for the os calcis and for the olecranon. According to the illustrations of Todd, the development was approximately that of 10 years and 6 months. The skull and sella turcica were normal. Osteoporosis was not noted. The general physical findings were quite normal. The blood pressure was 100/75. The mental development seemed average and the child did fairly well at school.

**Laboratory data:** The urine and blood were normal. The basal metabolic rate was minus 11 per cent. The twenty-four hour output of 17-ketosteroids in the urine was 1.3 mg. The normal range for this age is 1.5–6 mg. per 24 hours (Talbot). The urinary

gonadotropin assay was between 27.2 and 40.8 mouse units per twenty-four hours (high for this age).

Although absence of secondary sex characteristics in a girl 12½ years old certainly should cause no concern, the association here of stunted growth, complete infantile appearance, and high urinary gonadotropin titer, suggested that this patient might be a case of ovarian agenesis. Albright and his co-workers (1) stress the fact that the osseous development in these girls is only slightly retarded and that fusion of the epiphyses does occur in due time. On this basis, it should logically follow that any therapy directed toward the production of linear growth must of necessity be instituted early enough to allow for sufficient growth prior to epiphyseal closure. If, however, the therapeutic agent so employed simultaneously produces development of secondary sex characteristics, the optimum time for beginning treatment should be about the normal age of puberty. The patient had already had a therapeutic test of stilbestrol for two months at the age of 10½ years. She had responded with a spurt of growth as well as beginning sexual development. Therefore prompt verification of the diagnosis seemed desirable in order that precious time would not be wasted, and surgical exploration was made January 4, 1946.

**Operative report:** The abdomen was opened through a midline incision and the pelvic organs exposed. Filmy adhesions extended from the fundus of the uterus to the upper portion of the bladder. The uterus was undeveloped; its greatest width at the site of the tubal insertion measured 1.5 cm., its length 2 cm., and its thickness 0.5 cm. The tubes were infantile and markedly convoluted; the fimbriated ends were patent. The most striking feature was the absence of ovaries. These structures were replaced by narrow streaks of glistening, greyish-white tissue, approximately 3 cm. in length, 0.4 cm. in width, and 0.3 cm. in thickness, which showed no evidence of follicular activity. The appearance of this tissue was so characteristic and so similar to the illustrations of Wilkins and Fleischmann (33) that no biopsy was taken. A scarred appendix was removed.

**Subsequent course:** Nine days postoperatively, January 13, 1946, therapy was begun with oral stilbestrol, 0.5 mg. daily for three weeks, followed by a rest of one week. This was a deviation from our usual course which consists of 42 mg. of stilbestrol monthly, given as follows: 1.0 mg. daily for the first week, 2.0 mg. daily for the second week, and 3.0 mg. daily for the third week, with a rest interval of one week. The smaller dose was prescribed because production of withdrawal bleeding was not contemplated. Surprisingly, however, this dose was not small enough to prevent withdrawal bleeding from a uterus which, less than five weeks previously, was observed to be completely rudimentary. During the second month the dosage of stilbestrol was cut in half (5.25 mg. in three weeks). Withdrawal bleeding again occurred. The dosage was then reduced to 0.1 mg. daily for twenty-five days each month, and withdrawal bleeding continued to occur cyclically each month even on this small dose. After five months of therapy, the patient had grown 1.0 inch in height. There was considerable increase in the size of the nipples and areolae, and axillary and pubic hair had begun to develop. The uterus and cervix were still tiny. Our goal at that time was to produce maximum growth with minimum sexual development.

**Comment:** Although the effect of estrogenic therapy on the development of secondary sex characteristics and the production of withdrawal bleeding, when given cyclically, is well known, much interest hinges, in this case and others like it, on the increment of growth which may be

achieved thereby. The average height to which these girls grow is 55 inches. Will estrogenic therapy, given in time, improve on nature's results?

II. PRECOCIOUS PUBERTY

CASE RECORD

*M.L.*, a six-year old girl, was brought to one of us (M.B.G.) December 9, 1943 for consultation because of vaginal bleeding of eleven days' duration. The mother reported that the child had not been feeling well for several weeks and had lost her appetite. She had complained, November 28, of left-sided abdominal pain with some radiation down the left leg. That evening slight vaginal bleeding was noted and this continued, necessitating the use of two pads per day for the ensuing eleven days. The mother had noted the presence of some pubic hair about three weeks before, but had been quite unaware of beginning breast development. The child was described as a bright pupil, but rather irritable and quick-tempered. The family history is of interest for the following points: The mother had three pregnancies of which the first was normal. That child is living and well, age 13 years, the menarche having occurred at the age of 12½ years. The second child died of a blood dyscrasia at the age of two days. The third pregnancy (the patient) was complicated by nausea and vomiting throughout the nine months, but the child was born apparently normal and well. The mother was ill for about four years following this delivery, the major difficulty being a severe hypotension. The father is living, but is beginning to develop some type of neuromuscular difficulty involving one upper extremity. The maternal grandfather was diabetic. One maternal cousin menstruated at the age of 9 years.

The early development of this child was normal. She weighed 7 pounds and 4 ounces at birth, and she teethed, walked and talked at the normal times. At two years of age she developed pyelitis. This was followed by measles and recurrent pyelitis. Following the measles she developed diplopia, and strabismus was then noticed for the first time. At 3½ years of age she was quite ill with chickenpox, although no undue lethargy or somnolence was noted. She had tonsillitis, otitis media and cervical adenitis, for which an adenotonsillectomy was performed at the age of 5½ years (July 1943).

Figure 2a is a photograph of the patient at the age of six years. Her measurements were as follows:

Weight.....	59½ pounds
Height.....	47½ inches
Span.....	47½ inches
Upper segment.....	24 inches
Lower segment.....	23½ inches
Ratio of upper to lower.....	1.01

The child, when first seen, was tall for her age, well-developed, well-nourished and well-proportioned. She was somewhat unusual looking, due to the prominent frontal bosses and elongated mandible, giving an appearance suggestive of hydrocephalus. The scalp hair was abundant and normal in texture. There was an excessive growth of long hair on the forearms and legs, a slight amount on the pubis, but none in the axillae. The skin was soft, warm and smooth, but showed a definite tendency toward keloid formation. There were two small, flat, pigmented moles on the right side of the chest anteriorly, and one posteriorly. Dark shadows were present under the eyes. There was a bilateral internal strabismus, for which glasses were worn. The pupils reacted normally.

The fundi were normal. Perimetric studies were attempted but were unsuccessful. The dentition was normal for her age, as were all of the oral structures. The tonsils were absent. Small, shotty cervical lymph nodes were palpable bilaterally. The thyroid was normal. The breasts showed considerable rounding, and glandular tissue was definitely

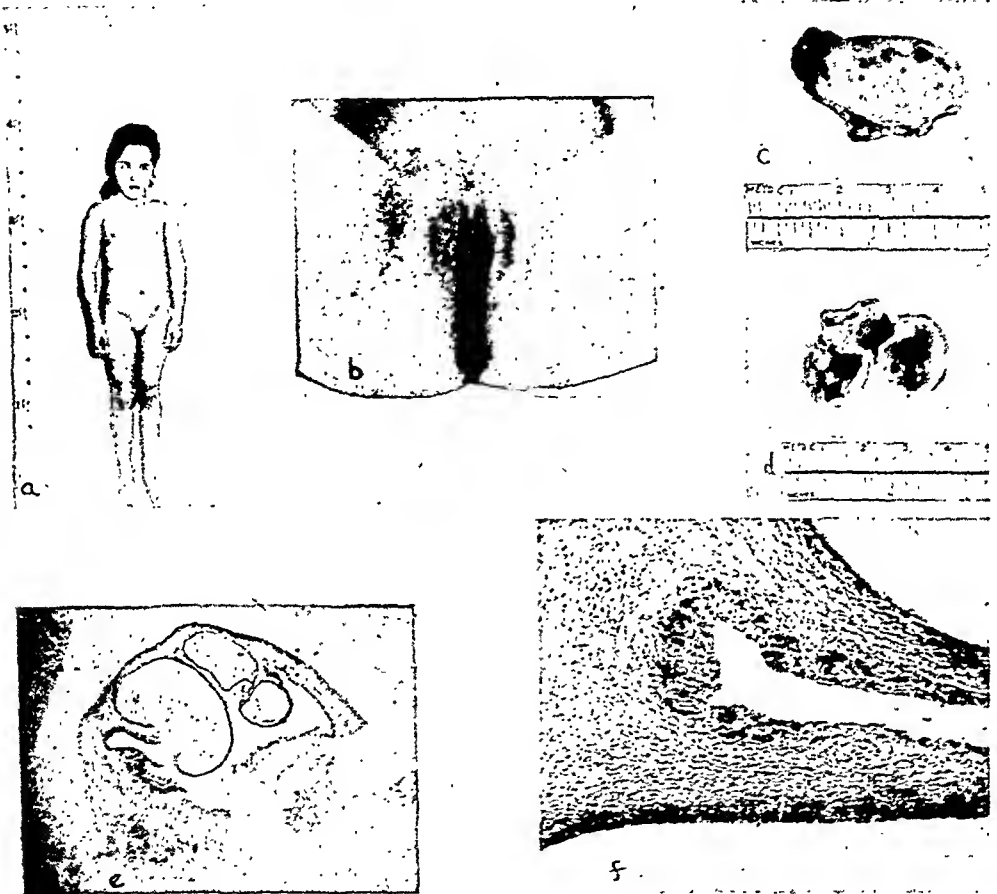


Fig. 2a. Age 6 years; height 47½ inches.

FIG. 2b. Note pubic hair and excessive labial development.

FIG. 2c. Cystic left ovary.

FIG. 2d. Appearance of left ovary on section.

FIG. 2e. Microscopic appearance of left ovary (×45).

FIG. 2f. Margins of three cysts: upper and lower lined by a thin layer of unluteinized granulosa cells; center lined by a thick layer of luteinized granulosa cells (×120).

palpable. The areolae were pigmented and showed increased development, as did the nipples. The heart and lungs were normal. The blood pressure was 100/60. The temperature was 99.6° orally. The abdomen seemed full, but no organs or masses were felt. On the left knee there was a shiny, raised, hypertrophic scar, about 1 cm. in diameter. The reflexes were hypoactive, the biceps and triceps not elicited. There were no pathologic



reflexes. The genitalia were moderately overdeveloped (Fig. 2b). The clitoris was hooded but not enlarged. Both labia majora and minora showed excessive development. The hymen was seen to pout, and bleeding was present. On rectal examination the whole pelvis seemed to be occupied by a large uterus. Nothing else could be felt. Vaginal smears stained by the Shorr technic showed large, clear, pink staining epithelial cells which were interpreted as signifying full estrogenic effect.

**Laboratory data:** The urine was normal. The blood showed an anemia. The hemoglobin was 71 per cent (10.2 gm.); the red blood cell count was 4,800,000, the white blood cell count 7,600, with 48 per cent polymorphonuclear cells, 49 per cent lymphocytes, 2 per cent mononuclear cells and 1 per cent eosinophils; there were 88 per cent filamented neutrophils and 12 per cent nonfilamented cells. The Wassermann and Kahn reactions were negative. Roentgenologic examination of the chest revealed moderately enlarged hilar shadows and slightly increased peribronchial markings. There was no evidence of a thymus or of a mediastinal shadow. The cardiac shadow was within normal limits with slight prominence of the pulmonic regions. Skull roentgenogram showed the calvarium to be rather thick, but normal in all other respects. The osseous development was that of an eight-year-old—two years' acceleration. Hormone assays were attempted; however, the collection of urine was so unsatisfactory that the results, as inaccurate, are not included.

Presented with the problem of differential diagnosis of female sexual precocity where there are no localizing signs, numerous possibilities require consideration. These possibilities were splendidly summarized by Novak (20) in a recent article. To the list of well-known causative factors such as adrenal, pineal, and ovarian tumors, and cases of cerebral origin, Novak added a new category, the so-called constitutional type. Unfortunately, this paper appeared a month too late for the latter diagnosis to be considered in our patient. The possibility of either an adrenal tumor or a pineal tumor seemed most remote. As pointed out by Reilly, Lissner and Hinman (22), adrenal tumors are nearly always virilizing, producing pseudosexual precocity in girls, and are rarely associated with precocious menstruation. Of the 177 cases of pineal tumors collected by Bing, Globus and Simon (3) up to 1938, only 56 were in children under 15 years of age, and of these 21 showed *pubertas praecox*. There was not one female in this group. Since the evidence is accumulating to suggest that pineal tumors *per se* do not cause sexual precocity, but do so only as a result of impingement on the posterior hypothalamus and the floor of the third ventricle, the cases of cerebral origin came up next for consideration. Weinberger and Grant (32) described 17 cases due to tumors of the hypothalamus. Of these, three were females. Other lesions such as hydrocephalus (Dorff and Shapiro (7)), encephalitis, and measles encephalomyelitis (Ford and Guild (9)), have been described as producing sexual precocity. Of the 544 instances of *pubertas praecox*, other than those due to pineal tumors, collected by Bing, Globus and Simon (3), 13 of the verified cases had cerebral lesions and 51 of the 440 clinical cases had histories of involvement of the central nervous system.

The possibility that our patient belonged to this group had to be weighed on the basis of her barely-suggestive hydrocephalic appearance, and the strabismus. The Albright syndrome came in for brief notice, only to be eliminated as none of the associated characteristic findings were present, namely, osteitis fibrosa cystica or disseminata, and cutaneous pigmentation. An ovarian origin for the sexual precocity seemed most likely, despite the lack of palpable evidence. In the 104 verified cases of Bing, Globus and Simon (3) 42 (40 per cent) were due to ovarian tumors. When it is considered that the 104 cases represented both sexes, the percentage of ovarian tumors as a cause of female pre-

cocity becomes more impressive. On the other hand, the incidence of granulosa-cell tumors as a cause of sexual precocity is not as frequent as we have been led to believe. Lull (18), up to 1941, was able to find only 16 such cases in the literature.

Because of the rapid development of the syndrome in our patient, the abdominal pain, relatively prolonged bleeding and anemia, it was felt that an ovarian tumor, possibly malignant, might be present. With this in mind an exploratory operation was performed December 17, 1943.

**Operative report:** The vaginal canal readily admitted the length of a finger. A well developed cervix was sounded for a distance of one inch from the external to the internal os; the uterus measured 7.5 cm. (3 inches). The cervical canal was readily dilated to size #8 Hegar. No endometrium was obtained by curettage, which was to be expected as the child had been bleeding for nineteen days.

When the abdomen was opened, the uterus was found to be the size of an adult organ. The right ovary was small (1.5 by 0.5 by 0.5 cm.). No cysts were seen or felt in the cortex; the corresponding tube was infantile in appearance. The left ovary was enlarged (4.5 by 2.5 by 2.0 cm.) and showed evidence of much follicular activity; the organ was soft, yellow, with many small, bluish cysts, 4 to 5 mm. in diameter, lying in the cortex, and a large cyst, 2.0 cm. in diameter, occupying the outer pole. The left tube resembled a normal adult oviduct. The left tube and ovary, and appendix were removed.

**Pathologic report:** The left ovary was 4.0 to 2.2 by 1.8 cm. in size (Fig. 2c). The surface was smooth and white with numerous bluish cysts apparent below the surface. On section, the small cysts in the outer cortex were 0.2 to 0.5 cm. in diameter and contained clear, colorless fluid (Figs. 2d and 2e). At a deeper level was a larger cyst, 2.0 cm. in its greatest dimension, with an irregular outline, also containing colorless fluid and having a pale yellow lining. The left fallopian tube was 3.5 cm. long and 0.5 cm. in diameter in the ampulla. It resembled a normal adult tube.

Microscopically most of the small cysts in the ovary were lined by two or more layers of granulosa cells; a few were lined by theca interna cells (Fig. 2f). The theca interna around these cysts was moderately vascular; small groups of theca cells were rounded and contained small vacuoles. The larger cyst was lined by a thick layer of large luteinized granulosa cells. The theca interna was vascular; its cells were mostly spindle-shaped, but in small groups they were rounded and vacuolated. The primary follicles and follicles in the early ripening stages appeared normal; the ovum in a later ripening stage was degenerated. There were numerous atretic follicles but no corpora lutea. In the fallopian tube, the mucous folds were thin, delicate, and divided into many small branches. The epithelium was high columnar and the cells were crowded. Pathologic diagnosis: follicle cysts of the left ovary with one cyst showing marked luteinization; adult type ovary and tube; ovarian hyperfunction.

**Subsequent developments:** The child made an uneventful recovery except for keloid formation in the abdominal scar. All signs of precocious sexual development gradually regressed and no further bleeding occurred. When the patient was seen July 23, 1945, at the age of seven years and eight months, nineteen months postoperatively, she showed the following: height, 53 inches, an increase of  $5\frac{1}{4}$  inches; weight,  $72\frac{1}{4}$  pounds; blood pressure, 90/60; pulse, 98. The child looked well, the strabismus was less evident, and the breasts and genitalia had regressed completely. The uterus and cervix were small, there was no pubic or axillary hair, the remaining ovary was not enlarged and the vaginal smear showed infantile type cells. Due to an intervening vacation period, followed by a prolonged febrile illness diagnosed as primary atypical pneumonia, photographs were not taken and urinary gonadotropic assays were not secured at this time. The child was

not seen until February 7, 1946, twenty-six months postoperatively. She had grown an inch and a half in a little more than six months. She looked pale and was nervous and fidgety. Signs of beginning maturity were again perceptible: slight development of breasts and growth of vulval and pubic hair and slightly increased development of the labia but not to the same degree as prior to operation. The uterus and cervix were not palpably enlarged and no bleeding had occurred. The urinary gonadotropin titer was not increased, being negative for 9.4 mouse units, the lowest level tested. Urinary 17-ketosteroids were 4.0 mg. per twenty-four hours.

Because of the possible cerebral origin of the syndrome and especially on account of progressive development of peculiar personality traits, encephalography was recommended but refused by the family.

**Discussion:** The finding of a unilateral polycystic ovary as the cause of precocious puberty was a little surprising to us. In a survey of the literature, by no means complete, we have noted only three other such cases: a 22 month old child reported by Lull (18), a 4½ year old girl by Fischer (8), and a 5 year old girl by Mengert (19). The latter case remained well for a follow up period of three years following extirpation of the cystic ovary.

In our case the ovarian pathologic changes might have been caused by an intracranial lesion, although its unilateral character, the regression of the symptoms postoperatively, and lack of recurrence for at least two years seems to speak against it. Furthermore, in all the proved cases of sexual precocity associated with lesions of the third ventricle or of the hypothalamus, no such changes have been reported in the ovary. A large cystic ovary did occur in one case of Albright's disease but no luteinization was observed, Sternberg and Joseph (28).

Finally the question arises as to what relationship this case bears to the nine reported by Novak (20), in which not only precocious bleeding occurred, but ovulation as well. Seven patients of his series were laparotomized; and of these three had unilaterally enlarged ovaries showing follicular cysts. Is our patient a representative of this group, merely with more pronounced cystic changes in the ovary? Had Novak's article been available to us at the time of operation, we might have been content to take a biopsy and allow the ovary to remain *in situ*. Our conjecture would be, however, that this child would then have continued to have prolonged episodes of bleeding, rather than normal regular periods, and would have subsequently required surgical intervention.

### III. VIRILISM

#### CASE RECORD

V. McD., age 18 years, was first seen by us (A.F.M. and M.B.G.) in January 1945, because of primary amenorrhea. Her mother had matured relatively late, age 16 years, and her menses had always been irregular; her only pregnancy was complicated by hypertension and toxemia.

The patient was an eight month premature baby who weighed 4 pounds 11 ounces at birth. Until her seventh year she was very delicate, having many respiratory infections and several bouts of prolonged and unexplained fevers. After her seventh birthday she began to improve in health, though she remained somewhat delicate and underweight. She was always small, the smallest in her class, and when sixteen years of age wore size nine clothes. At this time sugar was noted in the urine, but since a fasting blood sugar determination was normal this finding was discounted. Acne had been present since the age of thirteen to fourteen years. In 1943, between the ages of sixteen and seventeen years, the patient was treated for amenorrhea for several months at a time with courses of triweekly injections of estrogens in oil, in doses of 10,000 i.u. The patient knows that she grew very rapidly in the year, and her breasts began to develop, but she thinks that the beginning breast development and spurt in growth antedated the estrogenic therapy. Be that as it may, a few months following the institution of treatment, she noticed a sudden development of excess hair on face and body. Medication was stopped in November 1944. The patient claims that there has been no increase in the hypertrichosis since that time. Polyphagia and some polydipsia had been noted for two months. Large amounts of urine were passed at a time, but there was no nocturia. Perspiration was not excessive, although the patient felt warm. Mentally she was quite alert and intelligent and had an excellent memory. Though usually calm, she had been quite irritable for the preceding few months. She did not suffer from headaches.

Figures 3a and b are photographs of the patient, age eighteen years and three months. Her measurements were as follows:

Weight.....	118 pounds
Height.....	66½ inches
Span.....	66 inches
Upper segment (pubis to vertex).....	32½ inches
Lower segment (pubis to floor).....	34 inches
Ratio of upper to lower.....	.96

She was a slender, pale, odd-looking young girl with an excessive growth of coarse, blonde, facial hair extending along the cheeks, down under the chin, and onto the neck. There was, in addition, hair on the upper lip, a masculine pubic escutcheon, hair about the areolae, and long, coarse, wiry hair on the forearms and lower extremities. Extensive acne was present on the face and back. The eyes were wide-set and prominent, although the lid apertures were narrow. Ophthalmoscopic examination revealed normal fundi; the visual fields were normal. The dentition was normal except for the absence of one lateral incisor which had been extracted. The chest was quite narrow and the entire figure somewhat boyish, although there was some development of the breasts. The lungs were normal. The heart was not enlarged. There was a rough systolic murmur heard at the mitral area which was not transmitted. The pulse was 76 and the blood pressure 110/70. No organs or masses were palpable in the abdomen. The hands were slender and tapering and showed a coarse tremor on extension. The lower extremities were not remarkable and the reflexes were normal. Pelvic examination showed well developed labia and slight enlargement of the clitoris. The uterus and cervix were small. The ovaries were bilaterally enlarged to about the size of small hen's eggs and were firm to palpation.

Laboratory data: The urine was normal except for the frequent presence of a yellow precipitate when tested with Benedict's solution. The blood showed: hemoglobin, 94 per cent (13.6 gm.), 4,600,000 erythrocytes, 7,400 leukocytes; the differential smear showed: 68 per cent neutrophils, 28 per cent lymphocytes, 2 per cent monocytes, 2 per

cent eosinophils; filamented neutrophils were 87 per cent, nonfilamented, 13 per cent. The color index was 1.02. The basal metabolic rate was minus 2 per cent. The glucose tolerance test on January 23, 1945 was as follows:

	Blood sugar	Urine sugar
Fasting	93 mg. %	neg.
$\frac{1}{2}$ hour	206 mg. %	neg.
1 hour	253 mg. %	2 plus
2 hours	333 mg. %	4 plus
3 hours	233 mg. %	4 plus

Urinary hormone determinations revealed: twenty-four hour output of 17-ketosteroids (ketonic fraction) 21.1 mg. (normal 5.6 to 9 mg.); twenty-four hour output of gonadotropins, follicle-stimulating hormones (F.S.H.), 34 mouse units (normal); the combined estrogen level was reported as moderately low (exact figure not available). Vaginal smears, stained with Shorr's trichrome stain, showed definite evidence of estrogenic activity. Roentgenogram of the skull revealed the sella turcica to be normal in size and contour. The calvarium was moderately thin and uniformly calcified, showing no defects. Intravenous pyelograms showed no evidence of adrenal enlargement.

**Clinical diagnosis:** virilism with primary amenorrhea, uterine hypoplasia, hypertrophy of the clitoris, heterosexual hypertrichosis, impaired carbohydrate tolerance, and bilaterally enlarged ovaries, but without obesity, hypertension or plethora.

**Pathologic differential diagnosis:** (1) ovarian hyperthecosis (most likely); (2) bilateral arrhenoblastoma; (3) adrenal rest tumors of the ovary; (4) adrenal cortical hyperplasia or possibly adenoma. Pituitary basophilism was considered unlikely.

A pelvic exploratory operation was performed February 26, 1945.

**Operative report:** The abdomen was opened through a midline incision, and the pelvic organs exposed. A small uterus of normal contour, two inches in length, was pulled to the left of the midline by a dense fold of peritoneum which extended from the upper portion of the bladder to the left infundibulopelvic ligament; the right broad ligament was thereby stretched. The ovaries presented the following interesting features: both were enlarged and resembled the smooth "oyster-like" polycystic structures described by Stein et al. (25, 26); both were unusually firm to palpation; they were gray but innumerable small telangiectatic blood vessels coursing through the cortex suggested considerable vascular congestion; the right ovary was slightly larger than the left and measured approximately 6 by 5 by 3 cm. There was no evidence of follicular activity. The tubes showed normal adult development. Because of the bilateral involvement of the ovaries it was thought advisable to remove the excessive tissue and to restore the normal contour. A wedge-shaped resection was done on the right side

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FIG. 3a. Age 18 years; note hirsutism and acne.

FIG. 3b.

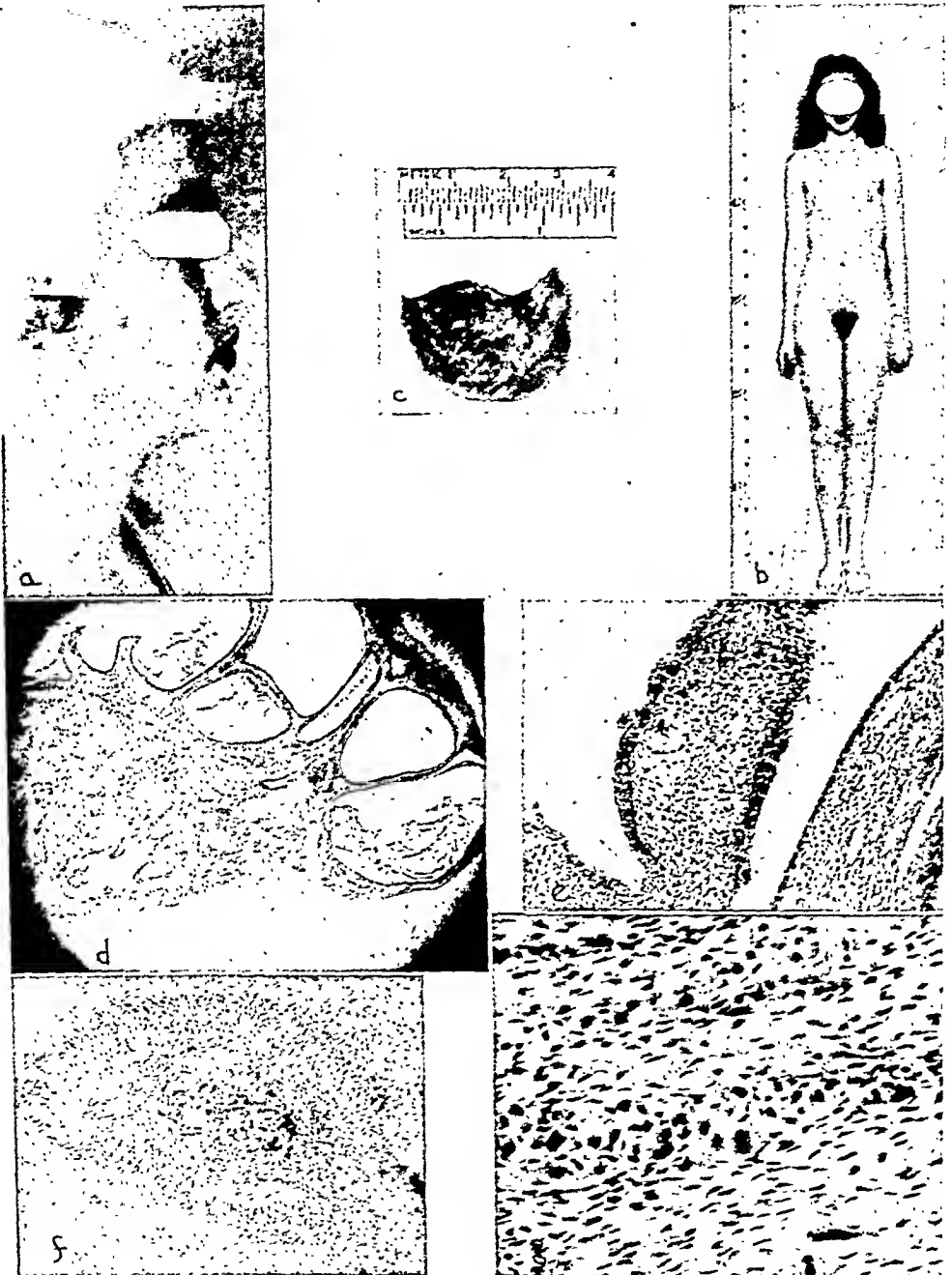
FIG. 3c. Resected portion of right ovary. Note cystic structure.

FIG. 3d. Ovary with follicle cysts in the cortex; thickened stroma with many atretic follicles ( $\times 30$ ).

FIG. 3e. Follicle cysts lined with unluteinized granulosa cells; hyperplastic luteinized theca interna ( $\times 120$ ).

FIG. 3f. Atretic follicle with central and peripheral masses of luteinized theca interna cells ( $\times 120$ ).

FIG. 3g. Strands of luteinized theca interna cells in the ovarian stroma ( $\times 500$ ).



FIGS. 3a-3g—See opposite page for description.

practically to the hilus; less tissue was removed from the slightly smaller left ovary. The unduly thick cortex offered unusual resistance to the scalpel. The cut edges were easily approximated with fine catgut sutures. Following resection, both ovaries were somewhat elongated but about normal in size. Exploration of the upper abdomen, including the adrenals, disclosed no abnormalities. The appendix was removed.

**Pathologic report:** The portion removed from the right ovary was 4.5 by 2.5 by 2.0 cm. (Fig. 3c), the one from the left ovary, 4.0 by 2.5 by 1.0 cm. The cortical surface was smooth and the tunica albuginea thickened, firm, white and opaque. The cortical layer immediately beneath it was filled with small cysts, 3 to 5 mm. in diameter, containing clear fluid; scattered similar cysts were present at a deeper level. The medullary part was unusually thick, firm and pale yellow.

Microscopically, the tunica albuginea was approximately three times the normal thickness (Fig. 3d). Small ripening follicles contained ova whose nuclei were degenerated, and small cystic follicles contained degenerated and collapsed ova. Atretic follicles were present in great number. No corpora lutea were seen, and in none of the cysts were the granulosa cells luteinized. The ripening follicles were surrounded by a wide zone of theca interna cells. Cells containing lipid were found around the larger follicles, around the cysts, and sometimes in the centers of atretic follicles and in strands and islands in the medullary stroma (Figs. 3e, f, g).

In frozen sections stained with Nile blue sulfate, no lipid substances were demonstrated the proliferating theca interna cells surrounding ripening follicles, but around the cysts the cells contained minute, pale, blue-staining granules. In the stromal strands and around the atretic follicles the cells contained small, spherical, deep-blue-staining granules, larger purple-staining granules and irregular red-staining clumps. Blue-staining with Nile blue sulfate is characteristic of soaps, fatty acids, cholesterol, cholesterol esters and lecithin, purples and reddish-blue probably representing mixtures of blue-staining and neutral fats. Unfortunately, material was not available for identification by the use of polarized light. However, similar small spherical granules have been identified in other material, by the use of the Hoerr-Romeis staining technic as phospholipid or cholesterol. That a correlation exists between hormonal activity and lipid content, particularly phospholipid and cholesterol, has been demonstrated by Bloor, Okey and Corner (4), and by Boyd and Elden (5), in the lutein cells of the sow's ovary, and by Greenblatt, et al., (15), Traut, et al., (29), Wolfe and Neigus (34), and Banner and Dockerty (2), in certain hormonally-active human ovarian tumors. In this case it should be noted that the older theca lutein cells, those around older atretic follicles and especially in the stromal strands, apparently contained a fairly large percentage of neutral fats, an indication that their hormonal activity was slight or absent. Ponceau-fuchsin staining failed to show the granules said to be characteristic of androgenic cells.

The resected portion of the right ovary was placed in butyl alcohol at the time of operation. It was subsequently sent to Dr. Herbert Evans' laboratory, at the University of California in Berkeley, where it was assayed for androgenic activity. The extract was evaporated at 56° C. and the residue dissolved in 2 cc. of sesame oil. One cc. was injected into each of two 21 day old hypophysectomized male rats six days postoperatively. No effect was produced on the seminal vesicles or prostate, indicating the absence of male sex hormone in any appreciable amount in the ovary.

**Subsequent developments:** April 3, six weeks after the surgical intervention and without any other treatment, the patient had her first menstrual period. These have since recurred at fairly regular intervals and have been characterized by normal flow with a duration of four to six days. Dysmenorrhea occurred on two occasions. The psy-

chologic effect of the establishment of normal menstruation has been dramatic. There have been no perceptible changes in the breasts, clitoris, labia or uterus. The acne is slightly improved while the hirsutism, though still present, is somewhat diminished, the hair being of definitely finer texture. Carbohydrate tolerance seems to have shown a steady improvement (Chart II). A glucose-insulin tolerance test, October 26, 1945, was interpreted as showing some degree of insulin resistance. The 17-ketosteroid assay of the urine had dropped from a preoperative level of 21.1 mg. to 16.8 mg. six months postoperatively, but March 30, 1946 had risen to 18.5 mg. per twenty-four hours (normal range 5.6 to 9 mg.). In April 1946 urine was sent to the laboratory of Dr. Herbert M. Evans for gonadotropin assay with partition of the F.S.H. and I.C.S.H. (interstitial cell stimulating hormone) fractions. Hypophysectomized female rats were injected with 120 cc. equivalent of urine. Neither follicular development nor repair of interstitial tissue was produced. The same amount was injected into hypophysectomized

V.M.D

## GLUCOSE TOLERANCE CURVES

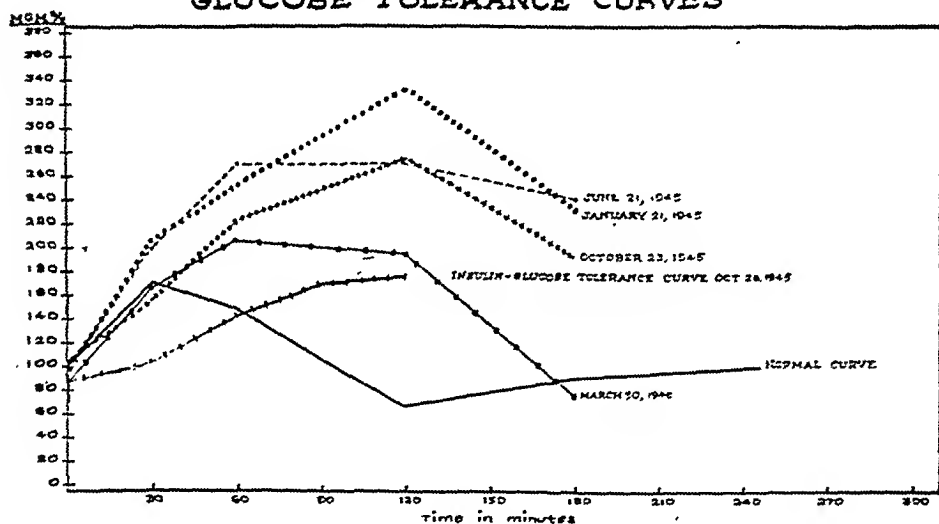


CHART II

male rats with no effect on seminal vesicles or prostate. It was concluded, therefore, that there were less than eight hypophysectomized rat units of either F.S.H. or I.C.S.H. fractions per liter of urine.

**Discussion:** Stein and Leventhal (26), in 1935, first insisted that the symptom complex of menstrual irregularities, notably amenorrhea, sterility, and varying degrees of defeminization or masculinization, in association with bilaterally enlarged cystic ovaries, constituted an endocrine syndrome. Up to 1945, Stein (25) had reported a series of sixty such cases, all treated surgically by ovarian wedge resection. In every instance this form of therapy resulted in the establishment or reestablishment of normal menstrual cycles and, in a significant percentage of cases, in the cure of



sterility. Stein and Leventhal (26), in 1935, stated that the only consistent pathologic change was the presence of follicle cysts lined by theca cells, while Stein and Cohen (27), in 1939, observed that luteinization of theca interna cells was the characteristic finding. Apparently, however, they did not consider these pathologic changes significant. They theorized that the polycystic changes in the ovary without corpus luteum formation were probably caused by an endocrine disturbance primarily hypophyseal, but that the resulting amenorrhea and sterility were caused by mechanical crowding of the ovarian cortex by cysts which interfered with the normal maturation of graafian follicles. It is on the basis of release of pressure that they explained the curative effect of ovarian wedge resection.

Fraenkel (10, 11), in 1941 and 1943, reported a similar instance in which removal of one enlarged ovary and partial resection of the other resulted in cure of amenorrhea, sterility and virilism. Examination of the removed tissue showed no clearly-demarcated tumor, but rather a diffuse hyperplasia of theca interna cells. Study of this case and of twenty-six others from the literature led Fraenkel to conclude that the syndrome was due to the hyperplasia of the theca interna cells of the follicles. He stressed the point that this thecosis bears the same relation to the thecoma as hyperplasia of the thyroid or pituitary glands, for example, bears to true adenomas of these glands. Furthermore, he stated that just as hyperthyroidism is amenable to surgical cure by subtotal resection of the hyperplastic gland, so may the symptoms of hyperthecosis of the ovary be cured by partial resection.

In our case we were pleased by the prompt initiation of normal and regular menstruation and the consequent psychologic improvement of the patient, but we were disappointed in the degree of regression of the hirsutism. This lack of regression apparently has been the general experience. At first we attempted to explain the results on the basis of persisting pathologic ovarian tissue, analogous to the situation where insufficient hyperplastic thyroid tissue is removed in hyperthyroidism. However, this explanation is untenable when one considers another group of cases, two reported by Geist and Gaines (12), two by Rottino and McGrath (23), and one by Cutting et al., (6), where bilateral ovariectomy was done and where little improvement in the signs of virilism ensued. Another explanation, therefore, must be forthcoming.

Normally, during the ripening of the follicle the theca interna cells proliferate and their cytoplasm becomes granular; a faint lipoid reaction can be demonstrated in them in the very early stages of corpus luteum formation. As the corpus luteum begins to degenerate, fatty acids appear in the cells of the theca interna. However, when a follicle undergoes atresia, the ovum and the granulosa cells perish, but the proliferation of

the theca interna cells continues. The proliferation is radial and eccentric and the cells, which contain fat at an early stage, become scattered among neighboring stroma cells.

Study of the microscopic pathology of our case leaves us with the impression that some factor as yet unidentified led to the premature death and degeneration of the ova, and that it is the increased amount of follicle atresia which accounts for the hyperplasia of the theca interna cells, most of which, on the basis of lipoid staining reactions, might be said to be hormonally inactive. Geist, et al., (13), and Greenblatt (14) showed that gonadotropins might produce this picture. Groher (16) demonstrated that androgens will stop development of normal follicles in immature female mice and that the follicles which developed prior to the administration of the androgens undergo degeneration and become atretic. In our patient the urinary F.S.H. titer was normal, the I.C.S.H. was not increased, but the 17-ketosteroid level was high. If the source of this high 17-ketosteroid titer lies in the adrenal cortex, as we must assume, is it intrinsic there or is it mediated through the pituitary-adrenocorticotrophic hormone? These are some of the unanswered questions, the final elucidation of which awaits a clearer understanding of the intricate pituitary-adrenal-gonadal relationships in the human subject.

#### SUMMARY AND CONCLUSIONS

Three nonneoplastic ovarian endocrinopathies have been reported.

I. Ovarian Agenesis. An 8 year old girl was observed for five years for failure to grow in height. Ovarian agenesis was suspected and verified by surgical exploration. An early diagnosis of this condition is of paramount importance so that valuable time is not lost during which epiphyseal closure renders linear growth impossible. The extraordinary potency of small doses of stilbestrol in producing development of secondary sex characteristics and withdrawal bleeding has been demonstrated. Sufficient time has not elapsed to evaluate the growth-producing effect of the drug in this case.

II. Precocious Puberty. A 6 year old girl with precocious sexual development and vaginal bleeding was thought to have an ovarian tumor despite lack of palpable verification. At laparotomy a unilaterally enlarged polycystic ovary was removed with prompt regression of all signs of precocity. After twenty-six months of remission, signs of ovarian activity again became perceptible. The possible cerebral origin of the syndrome is admitted. Encephalography was recommended but refused by the family.

III. Virilism. An 18 year old girl with primary amenorrhea, heterosexual hypertrichosis, slight enlargement of the clitoris, acne and impaired carbohydrate metabolism was found to have bilaterally enlarged ovaries.

Urinary assays showed high 17-ketosteroid and normal F.S.H. values. Ovarian hyperthecosis was suspected. A bilateral partial resection of the enlarged, firm ovaries was done with prompt establishment of normal menstrual function, improvement in the carbohydrate metabolism, and some regression, but not disappearance, of the hypertrichosis. The gross and microscopic pathologic changes are discussed. The patient's subsequent course corroborates the conclusions of both Stein (25) and Fraenkel (10, 11) that the treatment of choice at the present time is partial ovarian resection. It seems doubtful that the hyperplasia of the theca interna cells is of primary etiologic significance. The final elucidation of this syndrome awaits a better understanding of the pituitary-adrenal-gonadal relationships and the histogenesis and functions of the several ovarian cell types.

In conclusion, we believe that special emphasis should be placed on the psychologic aspects of these cases. The importance of making each one of these girls as nearly normal as possible for her age scarcely needs to be mentioned. Far-reaching psychologic trauma can thus be averted. In the first case, although fertility is out of the question, normal or near normal stature and secondary sex characteristics will make possible a normal life. In the second case, early psychologic trauma due to being different from other children, and the remote danger of early impregnation were obviated, while in the third case the personality changes which resulted from the mere establishment of the normal menstrual function were little short of a miracle.

#### ACKNOWLEDGMENTS

The urinary assays for gonadotropins and 17-ketosteroids were performed by Dr. Nellie Halliday of the University of California Hormone Laboratory, San Francisco, aided by a grant from Ciba Pharmaceutical Products, Inc., and the Breon Research Fund. The methods used were slight modifications of the Klinefelter, Albright and Griswold (17) technic for the gonadotropins, and the Pincus (21) method for the 17-ketosteroids. The preoperative gonadotropin assay on case III was done by Dr. Robert Lyon of the Gynecologic Hormone Laboratory of the University of California Hospital, and for the androgen assay of the resected portion of the ovary, and the later urinary assays for F.S.H and I.C.S.H. on the third case, we are indebted to Dr. Herbert Evans and staff of the University of California Institute of Experimental Biology, Berkeley, California.

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# PAROXYSMAL HYPERTENSION WITH CONCOMITANT SWELLING OF THE THYROID DUE TO PHEOCHROMOCYTOMA OF THE RIGHT ADRENAL GLAND. CURE BY SURGICAL REMOVAL OF THE PHEOCHROMOCYTOMA

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THE CLINICAL picture, pathology and treatment of tumors, originating from the chromaffin tissue of the adrenal medulla (pheochromocytoma), or from the genetically and morphologically identical chromaffin tissue adjacent to the sympathetic chain (paraganglioma), and their symptomatology, the suprarenal sympathetic syndrome, is well known today, Belt and Powell (7), Wells and Boman (46), and Howard and Barker (25).

The case we are about to report is unusual since the patient presented a symptom hitherto not described in the literature, i.e., paroxysmal swelling of the thyroid gland, accompanying and simultaneous with paroxysmal hypertensive crises. Furthermore, there were extremely rapid and excessive variations in blood pressure, which usually are not associated with the syndrome.

In 1934 Belt and Powell (7) reviewed the sixty cases already reported in the literature, and simultaneously presented a case of their own in which the pheochromocytoma, weighing 1000 gm. and containing 2 gm. of adrenalin per 100 gm. of tissue, replaced the right adrenal. In the same year Bauer and Leriche (4) reported the sixth surgically-cured case of a chromaffin tumor. This tumor was a paraganglioma situated close to a normal left adrenal gland. During the last decade the number of diagnosed and successfully-treated cases has risen considerably. Up to 1939, 103 chromaffin tumors were recorded, either diagnosed or found at necropsy, Brunschwig and Humphreys (12). Of these, 43 were found in the right adrenal, 34 in the left adrenal, 13 in both adrenals, and 13 were situated entirely outside the adrenal glands. In 1942, Kirshbaum and Balkin (27) collected 116 cases. In 1943, Hyman and Mencher (26) found a total of 35 surgically-treated cases of chromaffin tumor reported in the world's literature. Four of these were paragangliomas not involving the adrenal glands. The operative mortality in this series of 35 cases was 5. The tumor was malignant in only one patient who later died of metastases.

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## CASE REPORT

Mrs. B. B., 47 years old, housewife, was first seen May 26, 1945, by one of us, Bauer. For approximately two years she had been suffering from spells which lasted from five to ten minutes. At first the spells occurred every seven to ten days, and later became far more frequent. Finally she had several attacks each day, occasionally even at night. The attacks may be described as follows: the patient felt slight pain in the stomach, in the legs, and arms; she then developed nausea and felt a swelling in her neck anteriorly; her heart began to pound, she shook and experienced extreme weakness and fatigue; her skin became very pale, then red; her lips and fingernails became blue. After such an attack she perspired freely and felt tired and weak. The swelling in her neck persisted for at least one-half hour. The attacks usually occurred after breakfast but might occur any time, especially when she was fatigued. She lost seven pounds in weight during the two years following the onset of the attacks.

The patient was always healthy, her only illness being measles, in childhood. She was frequently constipated. Her tonsils and adenoids were removed at the age of 17 years. The menstrual cycle began at the age of 17 years; menstruation was regular and without abnormality. She had two children.

In her present illness the patient consulted several physicians. A heart specialist and an allergist found nothing abnormal. One rhinologist wanted to puncture a paranasal sinus, but another was against it. Another physician considered her to be neurotic. Later she was treated with theelin for menopausal disorders. Finally the patient's physician, Dr. Marian Goldwasser, observed her during an attack and noted that the systolic blood pressure rose within a few minutes from 110 to 200. This observation induced Dr. Goldwasser to refer the patient to Dr. Bauer.

**Family history:** The patient's mother died at the age of 48 years of diabetes mellitus. Her father died at the age of 76 years of unknown cause. One brother died at the age of 47 years from diabetes mellitus. Another brother, 62 years of age, is diabetic. Two brothers are living and well. One sister died at the age of 26 years following childbirth. Another sister is living and well at the age of 54 years.

**Physical Examination:** The patient was of normal body build; was 61 inches tall and weighed 127 pounds. She had prematurely grey hair. On routine examination, the pulse was 80, and the blood pressure 120/80 to 130/88. The thyroid was not enlarged. The lungs, heart, abdomen, and nervous system, did not show any abnormality. The lower pole of the right kidney was palpable. Pelvic examination did not reveal any abnormal findings. Fluoroscopy showed no abnormality in the chest. The urine did not contain any albumin or sugar.

With the tentative diagnosis of a chromaffin tumor, the patient was hospitalized for a few days in the Glendale Sanitarium, in order to study her during the attacks.

In the hospital several attacks were observed by the house physicians and nurses. During one attack the blood pressure rose from 120/80 to 230/40. Concurrently, a sudden rise in blood sugar was noted. During an attack June 5 at 11:30 A.M., the blood sugar was found to be 245 mg. per 100 cc. of blood, whereas the fasting blood sugar was 98 mg. per 100 cc. of blood. During another spell June 7, the blood sugar was found to be 150 mg. at 12:15 P.M., 160 mg. at 12:30 P.M., and 148 mg. at 12:45 P.M. During the attacks a marked swelling of the neck was noted in the region of the thyroid gland.

The patient was advised to have an x-ray study, with air insufflation in the area of the adrenals, and to undergo surgical treatment for the supposed chromaffin tumor. She requested, however, that a final effort be made to treat the condition medically. Although no hope of successful medication was held out by us, she was placed on pro-

stigmine bromide. Prostigmine was suggested because of its parasympathetic stimulating action, which conceivably might have counteracted the sympathetic stimulation experienced by the patient during an attack. However, oral administration of 15 mg. of prostigmine bromide three or four times each day produced no apparent improvement. The spells became more and more frequent, and August 18, one of us, Bauer, observed the patient during a typical attack. The course of the attack proceeded as follows:

The patient entered Dr. Bauer's office at 11:05 A.M., announcing the sudden onset of an attack. She felt extremely tired and weak; her heart was pounding; her arms, legs, back, and neck ached and pained; she felt a swelling of her neck; she was nauseated and shaking. The patient's face was pale; her hands showed a fine tremor; her thyroid was markedly enlarged, its contours being clearly visible. The whole gland was bulging; the skin over the frontal part of the neck was hyperemic; however, no thrill or bruit was found over the thyroid. The attack lasted approximately twenty minutes. The patient's fingertips and fingernails were slightly cyanotic at the end of the attack. The swelling of the thyroid disappeared completely in a few hours. The pulse rate at the onset of the attack was 84 and rose in 10 minutes to 96; the blood pressure was found to be 210/60 at 11:10 A.M. (five minutes after onset). It was taken at two to three minute intervals, and showed the following extreme variations during the next twenty minutes: 130/60, 240/75, 190/70, 100/60, 154/85, 100/70, 110/80. The blood pressure did not vary after 11:32 A.M. when the patient declared her attack was over. The pulse then was 80.

The patient now consented to an operation and she was seen August 10, 1945 by Dr. Belt. Urologic examination revealed the following:

An x-ray of the genito-urinary tract showed the kidney shadows to be normal in size, shape and position. A large round gallstone, the size of a quarter, appeared in the region of the gallbladder. The liver was normal in size. The iliopsoas shadows were clearly seen. By x-ray no mass was seen in either adrenal area. A number twenty-four cystoscope entered the bladder readily. Visual examination of the bladder revealed a normal mucosa. Both ureteral orifices were seen clearly, both functioned normally, and both were catheterized. Specimens from both kidneys were collected for Gram's stain and culture. Neither pus nor stainable bacteria were seen in either specimen of kidney urine; on culture both revealed gram-positive cocci. Retrograde pyelograms revealed a normal kidney pelvis on the left, with normal calices and a normal ureter pursuing a normal course to the bladder.

In the standing position the left kidney did not descend materially. It emptied promptly into the bladder. On the right side there was a hydronephrosis, Grade I, on the basis of I to IV. The ureteropelvic juncture showed an obstruction approximately 3 cm. below the renal hilus. From this point upward the ureter was somewhat dilated, a dilatation which extended to the renal calices. In the standing position this kidney descended a distance of 2 to  $2\frac{1}{2}$  cm., and in descending caused the ureter to kink 3 cm. below the right ureteropelvic juncture. In the retrograde pyelograms, displacement of the right kidney was very marked; it occurred only when the patient was in an upright position. In the roentgenograms taken without air insufflation around the adrenals, no definite evidence of an adrenal tumor could be discerned on either side.

The patient returned to the office August 18 for further study. At this time 60 cc. of air was injected into the perirenal space posterior to the kidney on each side. Diodrast was administered intravenously to bring out the shadows of the kidney parenchyma. A series of x-ray pictures was taken, first in the horizontal and then in the vertical position, in order to demonstrate the adrenal area. The left adrenal area was sharply defined and appeared to be normal. The right adrenal area was also clearly demarcated. At the

upper pole of the right kidney, extending upward toward the diaphragm, a tumor mass was seen which was the size of a golf ball. This growth was seen to move with the kidney and was interpreted as a right adrenal tumor.

**Treatment:** The tumor was surgically removed August 31, 1945. Under spinal anesthesia, the patient was placed in the overhand-swimming position with the right side up. An incision through the skin was made between the last two ribs. The periosteum of each rib in turn was exposed. It was incised along the middle of the outer aspect of the rib, and each rib was lifted out of its periosteal bed. The pleura was elevated carefully, the crus of the diaphragm was retracted upward, and a round, tense, cystic adrenal mass was exposed. It was covered with a network of distended veins. The vessels of the pedicle were ligated one after another, and just as the base was reached the cystic mass suddenly ruptured. Its content of brownish fluid, glistening with crystalline flakes of hemosiderin, filled the wound and was picked up with a sponge. The shell of active pheochromine tissue was then lifted easily from its bed. Complete hemostasis was assured and the wound was closed, leaving in place only one small rubber drain. During the period of operation no hypertensive crisis occurred. The patient made an uneventful recovery, was up on the third postoperative day, and left the hospital on the tenth postoperative day. She had no further attacks, although for a time the pulse and blood pressure showed moderate variations, both spontaneous and provoked by changes in posture. These variations were not associated with any discomfort and gradually subsided. Subsequently, October 9 and 16 respectively, the following pulse rates and blood pressures were recorded:

	Recumbent	Sitting	Standing
Pulse	80	84	96
B.P.	150/90	140/90	140/95-130/100
Pulse	68	72	80
B.P.	145/90	130/80	130/100

The tumor tissue removed at surgery was examined by Dr. Reuben Straus, pathologist of the Cedars of Lebanon Hospital. He reported as follows:

**Gross description:** The specimen consists of a newly opened cystic structure measuring approximately  $6 \times 6 \times 5.5$  cm. in diameters. It weighs 31.3 gm. The external surface is roughened in several areas by fibrous bands, and further presents several portions of bright yellow tissue having the color of adrenal cortex. This is present on two aspects of the mass, and is generally flattened. It appears compressed, so that only small ribbons of tissue traverse the external surface of the mass. The external surface is gray in color and presents a few areas of hyperemia. The wall of the cystic structure varies in thickness from about 2 mm. to a maximum of 11 mm. The maximum thickness is reached in the area of a smooth, rounded eminence, which projects into the cystic cavity. This eminence measures  $3 \times 3$  cm. in transverse diameters. On section this eminence presents a pale, glistening, homogeneous, apparently cellular surface, the appearance of which is not unlike that of a lymphosarcoma. The lining of the cyst, which generally is not smooth but glistening, is pale tan, although areas of dark brown discoloration are seen.

**Microscopic description from seven sections, suprarenal gland:** Six irregular pieces show portions of a cystic tumor mass. The inner surface of the cyst has no characteristic lining. Slight hemorrhage is noted here. The tumor is composed of large, irregular, poly-





FIG. 1. Low power view ( $\times 100$ ) of margin of tumor.

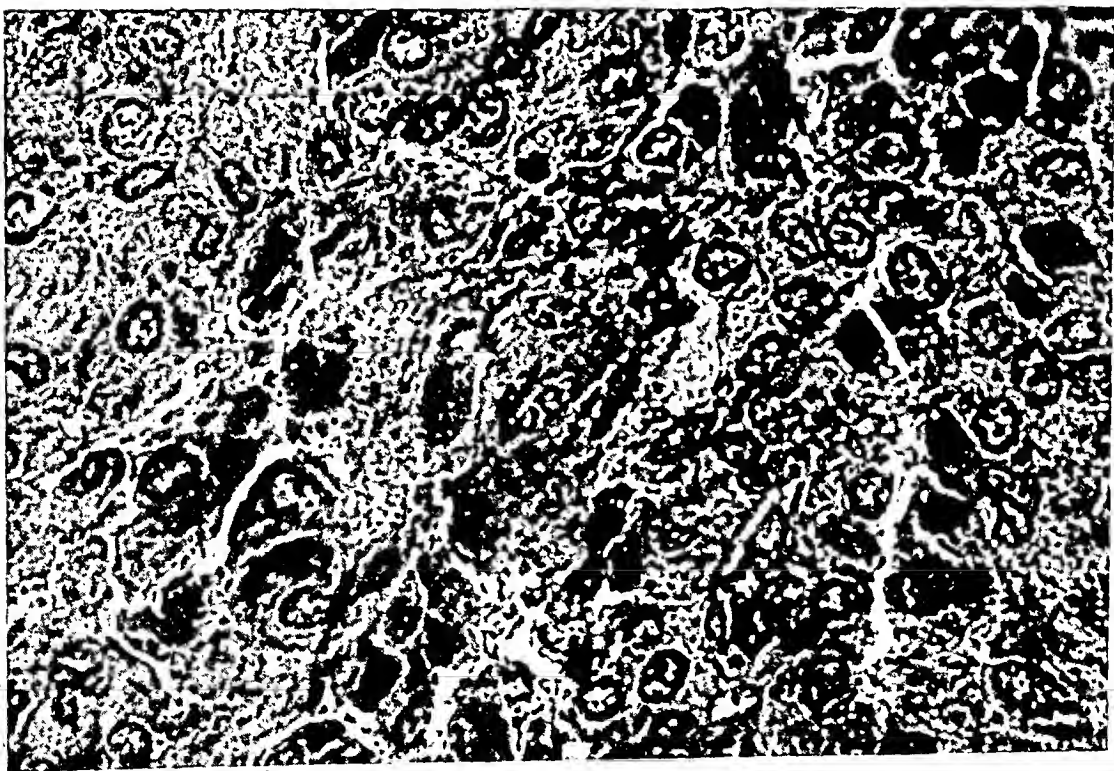


FIG. 2. High power view ( $\times 450$ ) of tumor cells. Dark brown granularity in the cytoplasm of some of the tumor cells.

bedral cells. The cytoplasm is abundant and lavender stained. The nuclei are small, fairly uniform, and some are hyperchromatic. The tumor is well vascularized. The external surface is bordered by a thick fibrous capsule. One of the pieces of tumor has a small amount of suprarenal cortical tissue, showing to a large degree compression atrophy and replacement fibrosis. Additional sections of the tumor, fixed in Zenker, Helly and Zenker's acetic acid, show a diffuse brown discoloration of all the tumor cells. Certain of the cells, however, show a distinctly darker brown granularity of the cytoplasm. Penetration of the tumor by the bichromate is somewhat uneven.

*"Diagnosis: Cystic pheochromocytoma of the suprarenal gland."*

### DISCUSSION

Several problems arise in hazarding a diagnosis of chromaffin tumor. We shall discuss a few of these in some detail, with a brief note on the treatment, pathogenesis, and pathology of the tumor itself.

**Diagnosis:** In our case the tentative diagnosis of a chromaffin tumor was based on the patient's description of her typical attacks, and on the fact that these attacks were associated with paroxysmal arterial hypertension. It was corroborated by the finding that paroxysmal hyperglycemia accompanied the attacks. Hence, the adrenalogenic nature of the disease was almost assured, since the attacks simulated entirely the effect of an intravenous administration of adrenalin (epinephrine). The diagnosis was established when the supposed tumor was visualized in the x-ray plate by perirenal insufflation.

Paroxysmal arterial hypertension is not necessarily indicative of the presence of a chromaffin tumor. Two monographs exist on paroxysmal hypertensive crises, the first published in 1905 by Pal (35) of Vienna, the second in 1934 in Paris by Bernal (8). Such crises have been observed in eclampsia, lead poisoning, essential hypertension, nephritis, aortitis, and various diseases of the nervous system such as epilepsy, traumatic or vascular damage to the brain, or meningitis. In a case of subacute meningococcic meningitis reported by Weber (45), paroxysmal elevations of systolic blood pressure from 130 to 240 were observed. In a case of tabes, studied by Rowntree and Ball (40), paroxysmal elevations of systolic blood pressure from 90 to 235 occurred. The supposed adrenal tumor was found neither at operation nor at autopsy. Hypertensive crises may accompany a diseased gasserian ganglion, Trémolières and Vêran (42), a sciatic neuritis, or a compression of the vagus by tumor masses, Donzelot (20) and Villaret (44). Vascular hypertensive crises have been reported in rare cases of typhoid fever and other acute infectious diseases. Monier-Vinard (31) observed paroxysmal hypertension caused by an allergy to Madeira wine. A patient reacted regularly to a glass of Madeira wine with a paroxysmal rise of his blood pressure from 160/90 to 280/140. This allergic reaction was provoked many times during a long period of observation.

These different varieties of paroxysmal hypertensive crises are probably brought about by a direct central or reflex stimulation of the medullary vasomotor center. This may, or may not, involve excessive liberation of epinephrine by central irritation of the splanchnic nerves. In a hypertensive crisis, if the diastolic pressure is only slightly elevated or not elevated at all, or if it drops even below the original level, then we are justified in presuming that the effect is due to the action of epinephrine. It was shown in 1912 by one of us, Bauer (2), that epinephrine, administered subcutaneously, may lower the diastolic pressure. Occasionally it may lower the pulse rate, probably by central stimulation of the vagus.

**Paroxysmal hyperglycemia** may or may not accompany paroxysmal hypertensive crises. Bauer and Leriche (4, 5) first reported a case of chromaffin tumor in which paroxysmal hyperglycemia accompanied the hypertensive crises. During an attack the blood sugar rose to 235 mg. % and fell to the usual value of 96 mg. % after 2½ hours. Van Epps, Hyndman and Greene (43) reported a case in which the blood sugar rose from 110 to 273 mg. % during the hypertensive crises. This sign, however, is variable. It was absent in a second case of Van Epps, et al., as well as in a patient examined by Engel, Mencher and Engel (22). A patient of Mortell and Whittle (32) showed a rise of his blood sugar from 100 mg. % to 245 mg. % during a hypertensive spell. Cahill (13) mentions a decrease in blood sugar during an attack, "evidently from rapid use of blood glucose." He does not cite a particular case or give figures. This action certainly is not in accord with the known effects of hyperadrenalinemia, nor does it conform with the mild transitory glycosuria observed in some cases after a hypertensive crisis. Paroxysmal hyperglycemia and transitory glycosuria, accompanying hypertensive crises, are not confined to chromaffin tumors, but may occur in other diseases. Therefore, paroxysmal hyperglycemia alone does not prove the presence of a chromaffin tumor.

**Provocation of hypertensive attacks** by any mechanism which calls forth an adrenalin discharge may be suggestive of the presence of a chromaffin tumor. Hyman and Mencher (26) enumerate the following causative factors of hypertensive attacks: emotional upsets, fear, anger, slight trauma, change in posture, massage on the side of the tumor, administration of histamine Roth and Kvale (39), or adrenalin, or immersion of the extremities in cold water. Artificial provocation of an attack did not occur in our case or that of Bauer and Leriche (4, 5). In the case of Belt and Powell (7), however, the tumor mass must have been compressed when they placed the patient on the surgical table in the overhand-swimming position and raised the kidney lift, thus provoking an attack of hypertension which terminated in death.

**Demonstration of adrenalin**, or at least of a pressor substance in the

blood of a patient during a hypertensive crisis, also may be suggestive of the presence of a chromaffin tumor. By chemical analysis Ernould and Picard (24) found increased adrenalin in the blood of such a patient. Beer, King and Prinzmetal (6) demonstrated the presence, during a hypertensive crisis, of a pressor substance in the blood of a patient with a chromaffin tumor. They used a modification of Pissemki's (37) method (perfusion of the denervated rabbit's ear). Since in their experiment the pressor effect of the blood was suppressed by ergotamine tartrate, it is almost certain that during the hypertensive crisis adrenalin was circulating in the blood in excessive amounts. In a case reported by Hyman and Mencher (26), (case 3), a pressor effect of the patient's blood during a hypertensive crisis was demonstrated on a dog's tail preparation; and an adrenalin-like effect was also obtained on a denervated cat's eye. A less delicate method of detecting adrenalin, by perfusion of a frog's leg, failed to give a positive result in the case of Bauer and Leriche (4, 5).

Demonstration of a vasopressor substance in the blood of a patient during a hypertensive crisis, however, is not necessarily indicative of the presence of a chromaffin tumor. Brandt and Katz (11) found such a pressor substance in the blood during hypertensive crises in a number of cases of paroxysmal hypertension due to causes other than chromaffin tumors. The following pharmacodynamic tests were employed, indicating that the pressor substance was actually adrenalin: (1) Pissemki's (37) method of perfusion of a rabbit's ear, (2) registration of the movements of an isolated rabbit's intestines, and (3) the mydriatic effect on an isolated frog's eye. All of these tests indicated that the pressor substance actually was adrenalin, the effects of which were abolished by ergotamine tartrate. Bernal (8) transfused into a normal person 300 cc. of blood which was withdrawn from a patient while suffering from an attack of paroxysmal hypertension. He observed a pressor effect of the transfused blood, which did not occur when blood was taken from the same patient during a free interval or from other patients not suffering with paroxysmal hypertension. The patient, whose blood exerted a pressor effect during the hypertensive crisis was not, however, suffering from a chromaffin tumor but from chronic lead poisoning. Demole and Rutishauser (19) report an extraordinary case of paroxysmal hypertension occurring in a patient suffering from a tuberculous destruction of the left adrenal gland only. The systolic blood pressure rose during the crises from 140-170 mm. Hg. to 210-250 mm. Hg. At the same time the blood sugar rose from 91-100 mg. % to as high as 170 mg. %. Transfusion into a normal recipient, of the blood taken from this patient during a hypertensive crisis, markedly raised the recipient's blood pressure and blood sugar for a period of ten minutes. This reaction failed to occur if other blood was used for transfusion.

We conclude, therefore, that demonstration of an adrenalin-like pressor substance in the blood of patients during hypertensive crises may be an interesting corroboration of the diagnosis of a chromaffin tumor, but is nonspecific and, therefore, not diagnostic of the presence of a chromaffin tumor. For practical diagnostic purposes it may be omitted.

**Visualization of the tumor on the x-ray plate** is the most important diagnostic procedure in cases of chromaffin tumors. In some cases pyelography may show a displacement of the kidney by such a tumor, Belt and Powell (7). Perirenal insufflation introduced by Carelli (16, 17, 18) in 1921, and popularized in this country particularly by Cahill (14, 15), will in most cases enable us to ascertain the diagnosis, and at the same time permit the surgeon to proceed with assurance to extirpation of the tumor. If x-ray studies do not support the tentative diagnosis of a chromaffin tumor, however, the clinical diagnosis may nevertheless be correct. A small adrenal tumor, or one that lies outside the adrenal gland, i.e., a paraganglioma, may escape visualization in the x-ray even with the aid of perirenal air insufflation. In the case of Bauer and Leriche (5) for instance, the cherry-sized paraganglioma adjacent to the left adrenal gland probably would not have been seen even after perirenal air insufflation. Such paragangliomas may be located even in the thoracic cavity, Phillips (36), in which position they certainly would not be visualized in the x-ray picture.

X-ray study after perirenal air insufflation is therefore, an indispensable diagnostic procedure, but a negative result does not disprove the presence of a chromaffin tumor.

**Extremely rapid and excessive variations in the blood pressure** were recorded during a hypertensive paroxysm in the patient here presented. To our knowledge such an observation has been made only once hitherto. We saw the blood pressure shifting, within a few minutes, from 210/60 to 130/60, then again up to 240/75 and down to 100/60. Mortell and Whittle (32) noted shifting of blood pressure in five minutes from 300/180 to 160 systolic, returning again in the next five minutes to 300 systolic. It is hardly possible to decide whether the excessive output of adrenalin took place in intermittent pushes, or whether the blood pressure variations were due to rapid changes in the responsive threshold of the vasoconstrictors. In Engel, Mencher and Engel's (22) case also, there were striking variations in the blood pressure; however, they were not as rapid as they were in our patient, nor did they occur during one hypertensive attack. Following a severe hypertensive crisis of unusual length, their patient showed a serious fall in blood pressure to 76/60, a level which persisted for several hours. Since the fall was associated with hemoconcentration, tachycardia, and torpor, the authors suggest "epinephrine shock" as a manifestation of pheochromocytoma of the adrenal gland. They compare this effect with that following the

prolonged administration of large doses of adrenalin in animal experiments. In 1918 it was shown, by Bauer and Froehlich (3), that prolonged perfusion of a frog's leg with adrenalin in weak concentrations inverts the vasoconstrictor effect of various short stimuli into a vasodilator effect. Faradic stimulation of the lumbosacral plexus, adrenalin in high concentration, pituitrin, and strychnine were the stimuli used in the experiment. A similar mechanism may be operating to produce the extreme variations in blood pressure as noted in some patients with chromaffin tumors. Engel and Aring (23) observed tremendous variations in blood pressure in an 18 year old patient with a cyst in the thalamus. The blood pressure was once found to be 88/40, and a few hours later 180/130. Less than a year before death it was 220/160, and the next day 120/80.

The abnormal carbohydrate metabolism noted in some cases of chromaffin tumor may be the result of an inadequate adjustment between the adrenalin output and the requirements of the body. The glucose tolerance test on the patient reported by Beer, et al. (6), and by Hyman and Mencher (26), revealed extreme variations in blood sugar which rose from 65 mg. % to 240 mg. % in one hour, followed by a fall to 50 mg. % at the end of three hours. The fasting blood sugar of the same patient fell from 105 mg. % to 15 mg. % one and one-half hours after the injection of 10 units of insulin, the fall being accompanied by profound hypoglycemic shock. A patient reported by McCullagh and Engel (29) showed similar unusual blood sugar variations in a glucose tolerance test, fluctuation extremes being from 128 mg. % to 267 mg. %, then down to 45 mg. %. In the case of Mortell and Whittle (32) the glucose tolerance test showed a shifting from 163 to 253 mg. %, and in three hours, to 74 mg. %. In a remarkable case of pheochromocytoma observed by Thorne, Hindle and Sandmeyer (41), the glucose tolerance test following administration of 100 gm. glucose revealed a paradoxical result: the fasting blood sugar was 130 mg. %; after thirty minutes the level was 100 mg. %; after one hour it was 68 mg. %; after two hours it was 77 mg. %; and after three hours it was 85 mg. %. The authors do not comment on this very strange result. This case, incidentally, showed persistent hypertension, rather than paroxysmal hypertension, and was cured by surgical removal of the tumor.

Paroxysmal diffuse swelling of the thyroid, accompanying a hypertensive crisis, is an extraordinary feature in our case which, to our knowledge, has not been reported in the literature. It was obviously caused by an intense paroxysmal hyperemia of the thyroid gland. The close relationship known to exist between the thyroid gland and the adrenal medulla was emphasized by Belt and Powell (7) in the study of the reaction of pheochromocytoma upon the thyroid gland, as revealed in many of the cases presented in the literature. One can only speculate about the mechanism



of this unusual sign which occurred in our patient. It might be explained on the basis of some facts outlined by the physiologist, Rein (38), and his co-workers. These investigators found that the carotid sinus nerve exerts a permanent depressing action upon the vasomotor tonus of the thyroid vessels. Severing of the nerve leads to a diminished blood flow through the thyroid, even without an essential alteration in the blood pressure.

Electric stimulation of the nerve, as well as a sudden increase in pressure in the carotid sinus, however, results in a considerable increase in blood flow through the thyroid gland. Therefore, the sudden rise of blood pressure in the carotid sinus, during the hypertensive crises in our patient, may have caused the tremendous transitory hyperemia of the thyroid with consequent enlargement of the gland. The reason this sign never has been reported in other cases with the same disease may be due to two factors: (1) it may have escaped the attention of both the patient and physician, if it occurred only in a mild degree, or (2) it may have occurred only in certain persons in whom the carotid sinus nerve was particularly sensitive. It was emphasized by Bauer (2) in 1912 that individuals vary in their reaction to the administration of adrenalin.

The pathogenesis of the paroxysmal crises in chromaffin tumors is a matter of conjecture. It is an established fact that these tumors always contain excessive amounts of adrenalin, the release of which obviously must be responsible for the crises. Hypertension, therefore, is a problem of the outpouring of adrenalin rather than its manufacture. Mechanical irritation of the tumor by changes in posture or other factors is not a satisfactory explanation of periodic hypertension. Emotional excitement does not regularly precede the hypertensive crisis; nevertheless, it is evident that the physiologic mechanism which normally regulates the discharge of adrenalin is dramatically modified in these patients. The mechanism responsible for this change is undoubtedly nervous in origin, i.e., sympathetic impulses reaching the chromaffin tissue by way of the splanchnic nerves. Bauer (1) pointed out elsewhere that frequently we meet with hormonal disorders which result from changes in the mechanism of discharge rather than from changes in the production of the particular hormone. Thus a vicious circle is established by the excessive amount of adrenalin released by the chromaffin tumor, which acts as a powerful excitant to the sympathetic system. In turn, the parasympathetic system is stimulated with consequent nervous and hormonal (insulin) counteractions. Finally, the entire autonomic nervous control of the body is upset, resulting in the ultimate discharge of insulin which counteracts the adrenalin. Individual constitutional differences, in the response of the various organs involved, may explain the differing clinical pictures resulting from the action of chromaffin tumors.

Not all cases of chromaffin tumor present the classical picture of paroxysmal hypertension as we saw it in our patient. Some develop a more or less rapidly progressive, permanent arterial hypertension, resembling the "essential" variety of hypertension. According to Nuzum and Dalton (34), an analysis of the cases reported revealed that the paroxysmal type of hypertension occurred in 22 cases; the constant type in 19. In rare cases of pheochromocytoma permanent hyperglycemia and glycosuria are displayed, instead of paroxysmal hyperglycemia and glycosuria. Clinically, these closely resemble the usual variety of diabetes and may be associated with it, Biebl and Wichels (9); McCullagh and Engel (29). However, in these cases of hyperglycemia and glycosuria associated with an adrenal medullary tumor, the syndrome is relieved by removal of the chromaffin tumor, Duncan, Semans and Howard (21). These cases represent one variety of what Bauer (1) named "symptomatic" hypertension and diabetes mellitus, as contrasted with the far more frequent, constitutional (genetic) types of these diseases. In the first instance, hypertension and diabetes mellitus are but symptoms of the presence of the chromaffin tumor; in the second instance, they are disease entities as such.

In certain cases of pheochromocytoma, unusual spontaneous variations in blood pressure occurred which were not followed by the characteristic clinical manifestations. In cases associated with diabetes mellitus, extremely variable spontaneous blood sugar levels have been recorded, McCullagh and Engel (29). Spells of nervousness, weakness and profuse perspiration may occur, which are not associated with or caused by paroxysmal hypertension or by paroxysmal alterations in the blood sugar level, McCullagh and Engel (29). The basal metabolic rate may be elevated and may mislead the observer into making an erroneous diagnosis of hyperthyroidism. The imbalance of the autonomic nervous system, created by the excessive production of adrenalin and more specifically the dramatic discharge of this hormone, may result in quite different clinical pictures, depending on individual variations in the response of the effector organs.

The pathology of chromaffin tumors has been described by Belt and Powell (7), and more recently by Nettleship (33). It requires no further comment here. The cystic type of pheochromocytoma found in our case is infrequently reported in the literature. The sharply defined, smoothly rounded contours of the tumor, as shown in the x-ray plate, suggest a cystic pheochromocytoma. The cyst form is caused by necrosis of the central part of the large tumor.

There was no indication of malignancy in our patient. Malignant pheochromocytomas are rare. Up to 1942, McGavack, Benjamin, Speer and Klotz (30) collected from the literature only eight reports of malignant pheochromocytomas. These metastasizing malignant tumors do not pro-



swelling of the thyroid gland was observed. This swelling reached its peak, and faded away, concurrently with the rise and fall of blood pressure, in each hypertensive attack.

A cure of this feature and of the entire suprarenal sympathetic syndrome was effected by the surgical removal of the pheochrome tumor.

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# THE EFFECT OF THIOURACIL DERIVATIVES ON FETUSES AND INFANTS

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**G**RADUALLY, as derivatives of thiouracil become of general use in the treatment of hyperthyroidism, it proves necessary to find out whether cretinism in a light or severe degree can be induced in the fetus by administration to the mother's organism of an antithyroid substance.

Clinical experiences in this problem are scarce. Eaton (3) reported two cases in which thiouracil was given to pregnant women suffering from hyperthyroidism. One of these women bore a child whose thyroid was transitorily enlarged for some time after birth. The child was normal in all other respects. In the case of the other woman the thiouracil treatment was discontinued four weeks before parturition. This woman likewise bore a normal child. Palmer (7) treated with thiouracil three pregnant women suffering from hyperthyroidism. In one case the treatment was continued from the fourth to the ninth month; in other words, right up to parturition. The child was normal in every respect.

In the Bispebjerg Hospital, Medical Department B, a 36 year old woman was treated successfully for hyperthyroidism, through the first period of her pregnancy, with adequate doses of methylthiouracil. Because of complicating heart failure the fetus was removed in the fifth month of pregnancy. The thyroid gland of the fetus, which had a total length of 23 cm., was hyperplastic, weighing 335 mg. The average weight of the thyroids of ten normal controls of corresponding size varied from 150 to 260 mg.

Vogt (8) reported a 25 year old pregnant woman suffering from hyperthyroidism. She was treated with methylthiouracil, 600-100 mg. per day, until parturition. Although she herself developed a marked goiter during the treatment, there was no thyroid enlargement in her child.

The effect of thiouracil on pregnant rats and on the growth rate of immature rats has been described in detail by Hughes (4). Hughes induced a pronounced myxedema in infantile rats by administration of thiouracil from the first day of life. The cretinism manifested itself by retardation of growth and development of bones, a mild degree of anemia, great mortality, and development of thyroid hyperplasia. "The young of thiouracil-treated mothers appeared to be normal but showed evidence of thyroid hyperplasia as early as one day of age, and retarded development at ten

days, indicating the possibility of placental and mammary transmission of the drug. Cretinism was not seen if thyroxin was given concurrently." Even feeding thiouracil to hens may cause thyroid enlargement in chicks, Andrews and Schnitzler (1).

The question which must be of particular interest to the clinician is whether the thiouracil effect can injure the fetus in the uterus. Hughes' (4) experiments seem to show that the growing organism is very susceptible to a deficiency in thyroid hormone. As the thyroid hormone is generally supposed to be able to pass through the placenta from mother to fetus, the latter need not necessarily be subject to thyroxin deficiency even if it is exposed to thiouracil from the mother. In the treatment of hyperthyroidism, the dose of thiouracil is generally adapted to preserve an approximately normal thyroxin concentration.

During the treatment of hyperthyroidism in human pregnancy the fetus may be influenced by thiouracil if this substance really passes through the placental membrane. The extent to which the blocking of thyroxin production may be compensated for is dependent upon the super-normal production of thyroxin by the mother and the fetus itself.

As this problem could not be studied systemically in women, because of the possible consequences to the fetuses, we have studied the development of the fetuses and the young of rats and rabbits during the influence of 4-methyl-2-thiouracil administered directly, through the diet, or indirectly, transplacentally or transmammarily, by administration to the mothers. The details will be published in *Acta Pharmacologica et Toxicologica*, 1946.

## RESULTS

**Transplacental transmission of thiouracil derivatives.** The following experiment was carried out with the object of proving that thiouracil compounds are transmitted transplacentally from mother to fetus. Some normal pregnant rats were fed on the usual standard diet +20 mg. 2-thio-4-n-propyluracil per 10 gm. diet, for four consecutive days. The uterus was opened and the fetus removed, great care being taken not to establish a contact between the blood or tissue fluid of the fetus and those of the mother. Each fetus, which was nearly full-grown, was washed thoroughly and next dried at 100°C. The fetus was then given to normal rats, mixed in the standard food. These rats developed typical thyroid hyperplasia. On the basis of our knowledge of thyroid hyperplasia after administration of propylthiouracil we could estimate the antithyroid effect of each rat fetus to correspond to 0.01–0.1 mg. propylthiouracil. Normal rat fetuses were found to contain no goitrogenic substances.

**Effect of methylthiouracil in utero.** In order to study the effect of methylthiouracil on the fetus, we fed a group of pregnant rats on a standard diet to which was added 0.25 mg. methylthiouracil per 10 gm. diet. This amount

of methylthiouracil suffices to bring about marked thyroid hyperplasia in less than ten days, and it parallels the dose used in human hyperthyroidism. Methylthiouracil was given from the time of conception to the day of parturition.

Several litters of normal rats which were fed on the same standard diet but with no addition of methylthiouracil served as a basis for comparison. The thyroids of all rat fetuses examined were found to consist of a solid cell mass with a little connective tissue. The more developed fetuses presented beginning differentiation into solid cell cords, but no colloid. (On this point the development of the human thyroid differs from that of the rat. Within the latter half of pregnancy the thyroid of the human fetus has a histologically more normal appearance with well-developed follicles.) After a few hours of life newborn normal rats presented a beginning development of colloid substance. The follicles increased quickly in number and size. In 10-day-old normal young the cell height was greater and the amount of colloid relatively smaller than in mature normal rats. Not until about the twentieth day of life did the thyroids of the young assume the same histological picture as that of a full-grown normal rat. These observations accord with Kull's (6) description of the late embryonic development of the thyroid gland of the albino rat.

The young which had received methylthiouracil transplacentally showed at birth signs of the results of a slight antithyroid effect, since the thyroid contained no colloid but consisted exclusively of solid cell masses. Colloid appeared in these cases one or two days later than normally, but the further development of the thyroid corresponded exactly to that of the control animals. No increase in weight of the thyroids of the infantile rats could be observed or registered. It should be borne in mind, however, that the thyroid of the normal, newborn rat weighs about 1 to 1.5 mg. Thus only slight transitory histologic changes could be demonstrated. The further development of these young was exactly like that of normal young. This was also the case with the young whose mothers had received a considerable amount of methylthiouracil in their diet, throughout pregnancy.

**Transmammary administration of methylthiouracil.** When methylthiouracil was administered to suckling rats immediately after parturition, thyroid hyperplasia could be traced in the young after one week, and twelve to fourteen days after birth a maximal thyroid hyperplasia was found. Such young presented a slightly retarded growth rate, expressive of an artificial, infantile myxedema, even though the addition of methylthiouracil to the mother's diet was discontinued before the end of the period of lactation, ten days after parturition. The period of lactation is supposed to last about twenty days or more. The thyroid was regenerated completely within less than one month.

**Direct administration of methylthiouracil to young animals.** A consider-

able retardation of growth, together with the development of an infantile myxedema, was observed in the cases in which the direct administration of methylthiouracil was continued after the period of lactation. These observations correspond exactly to Hughes' (4) descriptions.

**Combined administration of methylthiouracil and thyroid hormone.** The thyroid hyperplasia generally brought about by administration of methylthiouracil to infantile rats could be prevented entirely by concurrent administration of thyroid hormone. Thus it is plain that the dangerous element for the fetus and the growing organism is not the thiouracil as such, but the low concentration of thyroxin in the tissues induced by this substance.

### DISCUSSION

The above experiments may illustrate to what extent the thiouracils are clinically applicable in pregnant and suckling women, and the information they give on the necessity of thyroid activity in fetal life is of physiologic interest. About the latter point there can be no doubt that the thiouracils can pass from the mother's organism into the fetus. Christensen (2) recently found that many of the antithyroid substances are highly protein bound when occurring in human plasma. Of 4-methyl-2-thiouracil and 4-n-propyl-2-thiouracil, only 35 and 8 per cent, respectively, were removable through ultrafiltration under corresponding circumstances. However, in our experiments this has not prevented sufficient amounts of the latter substance from penetrating the placental membrane. Considering the fact that the infantile growing rat is very susceptible to the influence of thiouracil, it is remarkable that the influence on the fetus seems to be rather small. This indicates that the thyroid hormone is not of vital importance for the course of the pregnancy and the intra-uterine development of the rat fetus. Attention should be called here to the fact that in the case of man the thyroid seems to function earlier in fetal life than in the case of rats. That the so-called congenital myxedema in man does not occur until a few months after birth, and that the thyroid of the fetus seems capable of reducing an existing myxedema in the mother, Zondek (10), are facts which argue greatly in favor of the theory that the thyroid hormone can pass through the placenta. Also the thyrotropic hormone is supposed to be able to pass through the placenta, Whiteside (9). When, therefore, a pregnant organism is exposed to the influence of a thiouracil derivative the fetus is hardly affected by the antithyroid substance as such, but the influence of this substance on the fetus probably depends on the effective amount of thyroid hormone present whether originating from the fetus itself or from the mother. Accordingly, when a pregnant woman suffering from hyperthyroidism is treated with a thiouracil derivative there should, theoretically, be no chance of injuring the fetus with the thiouracil as long as the

mother's metabolic rate is kept within normal values, but if the mother becomes affected during the treatment with an artificial myxedema, the fetus will share the same fate. For that same reason, overdosage during pregnancy is detrimental to the fetus. The use of thiouracil derivatives should be avoided entirely during lactation, because such drugs are so easily transmitted through the milk to the baby's organism, which is particularly susceptible to the antithyroid substances.

#### SUMMARY OF EXPERIMENTAL RESULTS

The experimental results obtained by Hughes (4), with 2-thiouracil, have been confirmed and extended through our experiments; 4-methyl-2-thiouracil administered directly to rats and rabbits could bring about pronounced cretinism in young animals or animals which received the substance through the milk.

Fetuses of pregnant rats which were given a diet containing 2-thio-4-n-propyluracil brought about thyroid hyperplasia in normal rats when the fetuses were mixed in the food of the latter. This was regarded as direct proof of the transplacental transmission of the thiouracil derivative from mother to fetus.

Rats which had been under the influence of methylthiouracil alone *in utero* showed evidence of only a transitory, slight thyroid hyperplasia, and no subsequent retardation of growth.

Administration of thyroid hormone concurrently with methylthiouracil prevented the development of infantile myxedema, as first described by Hughes (4). Thus it is not the thiouracil derivative but the thyroid deficiency that is detrimental to the quickly growing organism.

#### CONCLUSION

The probable conclusions of these results are that hyperthyroidism in pregnant women may be treated with thiouracils but overdosage should carefully be avoided; during lactation administration of thiouracils to the mother, or the lactation itself, should be discontinued.

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# THYROID AND ADRENAL INTERRELATIONS WITH SPECIAL REFERENCE TO HYPOTRICHOSIS AXILLARIS IN THYROTOXICOSIS

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FOR SEVERAL decades many investigators have been interested in the interrelations of the thyroid and adrenal glands. With alteration in the function of one of these glands, physiologic and anatomic changes have been observed in the other. However, the many contradictory conclusions that have been drawn indicate the great need for elucidation. Although definite progress has been made in the last few years, there are many phases of the problem that are as obscure as is the etiology of Graves' disease.

Since it was noted, in the course of examining thyrotoxic patients, that many of them tended to have less axillary hair than normal, and since the amount of axillary hair is governed to a large extent by the androgenic function of the adrenals (1, 6), the interrelationship of the two glands was given further consideration. The results are reported in this paper. Our interest in the significance of axillary hair as an indicator of the androgenic function of the adrenal glands was aroused in 1941 by Dr. Fuller Albright. Since then it has been the policy of the author to observe carefully in all patients the amount of axillary hair regardless of what their main complaint may be. These observations represent the standards for comparisons in the thyrotoxic patients.

## METHOD OF STUDY

Each of 102 thyrotoxic patients examined consecutively at the Boston City Hospital was included in the study. Without any preliminary explanation the patient was asked: "Do you have about the same amount of hair under your arms as does the average person?" If a subnormal amount of hair was observed, the patient was asked if it had always been less than normal, whether the amount had changed with the development of thyrotoxicosis, and whether any other members of the family had a subnormal amount of hair. When there was doubt as to whether there was a decrease in hair, the patient was credited as having a normal amount. The quantity of pubic hair was observed, but inasmuch as this hair is not as delicate as

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axillary hair as an indicator of decreased glandular function, most of the emphasis was placed on the changes in axillary hair. In determining whether the quantity of axillary hair present was normal, consideration was given to the sex, age and nationality of the patient, as well as other factors which might influence the amount of hair. In this connection, dam-



FIG. 1. Mother of several children, age 40, with moderate thyrotoxicosis; never had any axillary hair.

age of the liver was borne in mind, and in the last 70 of the 102 cases a record was made of the incidence of telangiectasia of the skin, liver palms, and gynecomastia.

### RESULTS

Of the 102 patients examined, 50 (49 per cent) had a subnormal amount of axillary hair; nine either had no hair or a very rare sprig (Figure 1; Table 1). Sixty per cent of these patients had noticed that the amount of their axillary hair was moderately or markedly less than normal; 40 per cent had observed slight or no change. Twenty subjects were confident that they had always had a subnormal quantity of axillary hair. Twelve patients stated that the amount of axillary hair had decreased with the development of thyrotoxicosis, but one individual thought that there had

been an increase. Six patients were confident that the quantity of axillary hair was subnormal in from one to four members of their family.

Forty-three (86 per cent) of the patients with decreased hair were females; in the group with a normal amount of hair, 73 per cent were females. The patients with decreased hair were from 19 to 72 years of age, averaging 45 years; in the normal group the average was 36 years. The amount of hair was not related to the duration or the severity of the disease. No significant changes in the head or body hair were observed.

Of the 50 female and 20 male thyrotoxic patients who were examined especially for telangiectases, lesions of the spider type were found in 16 individuals, 14 of whom were females. These lesions consisted of very small

TABLE 1. AMOUNT OF DECREASE IN AXILLARY HAIR

	No. of Patients
++++*	9
++++	8
+++	13
++	11
+	9

\* +++++ = None or very few hairs present.

+ = Slight decrease in amount of hair.

tortuous blood vessels which traversed each other, occasionally appearing to have a belly in the center. In some cases close inspection was necessary to locate the telangiectases. Simple linear dilations of the small vessels were not included because their significance is uncertain. Round, well-circumscribed, nodular telangiectases were noted to occur in 16 patients, but these were regarded as much less important than the spider-shaped ones. None of these vascular abnormalities was related to the age of the patient, the duration of the thyrotoxicosis, or to its severity. One individual, on becoming pregnant, experienced a great increase in the number of telangiectases.

Liver palms were noted in 16 subjects and gynecomastia in 3. One patient had hypotrichosis axillaris, gynecomastia, telangiectases of the skin, and liver palms. However, there was no clear-cut grouping of the disorders in the other cases. Some patients with very little axillary hair had no telangiectases, and the reverse situation was observed.

## DISCUSSION

The quantity of axillary hair is subnormal in association with several diseases. It is decreased, ordinarily, in patients with Simmonds' disease, Addison's disease, hypogonadism, myxedema, or cirrhosis of the liver, and sometimes it is subnormal in other chronic debilitating diseases. Rarely

patients are seen who say that they have never had very much axillary hair although there is no evidence of any of the foregoing diseases. In some instances it is stated that there is a subnormal amount of axillary hair in most of the members of the family. This condition may be the result of a defect in the end-organ, i.e., the hair follicles, rather than the amount of hormone produced.

In considering the etiology of the hypotrichosis axillaris in our patients with hyperthyroidism, the presence of panhypopituitarism, Addison's disease, and hypogonadism can be disregarded. Although none of the patients had frank cirrhosis of the liver many of them may have had impairment of liver function. Weller (14) found, at necropsy, pronounced parenchymatous hepatitis in 22 of 44 cases of Graves' disease. Moreover, Youmans and Warfield (15) observed that impairment of liver function was common in this disease. In studying many cases of cirrhosis of the liver, decreased axillary hair was often noted (7). The not infrequent coexistence of telangiectases and liver palms, as well as decreased axillary hair, would be in accord with impairment of liver function. These changes, and the gynecomastia that was observed in three cases, could be associated with a decreased supply of androgenic hormone and a decreased inactivation of estrogens. Whereas the hypotrichosis axillaris possibly was related to impairment of liver function in some of the thyrotoxic patients, this would not seem to be the main pathogenesis, since in most instances the subnormal amount of hair was observed by the patient before the manifestations of hyperthyroidism appeared.

The question may be raised as to whether the subnormal amount of axillary hair signifies a decreased adrenal function, or some constitutional defect which predisposes to the development of thyrotoxicosis. In this connection, some of the work of Marine should be mentioned. In some patients developing Graves' disease, he regarded the constitutional background as one of the most important etiologic factors (8). He considered Graves' disease as ultimately associated with "insufficiency of some secretion of the suprarenal cortex and gonads." He stated that the changes in the thyroid represented a compensatory reaction to the abnormalities in the adrenals or gonads.

In the rabbit and cat with an intact thyroid, a transient symptom complex was produced by injury to the suprarenals, which was considered as closely resembling Graves' disease (9). The outstanding changes were increased metabolism beginning between the third and sixth day and lasting from a week to several months, myasthenia, regeneration of the thymus, hypertrophy of the lymph glands, increased appetite, increased irritability, and hypersusceptibility to drugs.

Shapiro and Marine (11) reported that in 50 cases of Graves' disease

feeding of a glycerol emulsion of very fresh ox suprarenal cortex caused a striking gain in body weight and muscle strength. On the other hand, Weinstein and Marlow (13), using an adrenal cortical extract of greater potency, did not find that the course of hyperthyroidism was influenced by this therapy. Crile (2) believed that the adrenals were hyperfunctioning in Graves' disease.

Hoskins (5) observed that desiccated thyroid produced hypertrophy of the adrenal cortex in young guinea pigs. Thyroxin does not induce adrenal cortical hypertrophy in the hypophysectomized animal.

Removal of the thyroid gland hastens atrophy of the thymus gland, while removal of the adrenal glands causes regeneration of the thymus. In thyrotoxicosis, many of the changes in lymphoid structure are the same as those in adrenal insufficiency. There tends to be enlargement of the thymus and spleen, and lymph nodes, and there is a relative lymphocytosis in the blood. Adrenotropin (3), or adrenal cortical extract, when administered to rats, causes a decrease in the size of the thymus, lymph nodes, and spleen, and a lymphopenia. Since Cushing's syndrome is a result of hyperadrenocorticism, some of the changes in this disease may be compared with some of those in thyrotoxicosis. Weakness is generally present in each, as is a decreased glucose tolerance test. Osteoporosis is rarely found in thyrotoxicosis, but often found in Cushing's syndrome. In the latter, there is usually an increased excretion of 11-oxysteroids (12), and 17-ketosteroids (4). In thyrotoxicosis there may be a decreased excretion of 17-ketosteroids in the urine (4). In thyrotoxicosis some of the adrenal changes probably are manifestations of a chronic alarm reaction, Selye (10).

### SUMMARY AND CONCLUSIONS

A subnormal amount of axillary hair was observed in 50 of 102 patients 49 per cent, with thyrotoxicosis. In most of these cases the axillary hypotrichosis was apparently present before hyperthyroidism was manifested, and consequently the question is raised as to whether the decreased axillary hair is associated with some constitutional abnormality predisposing the individual to the development of thyrotoxicosis. The role of the adrenal glands and of the liver are given due consideration. Telangiectases of the skin and liver palms were not infrequently seen. The pathogenesis of these changes remains to be proven.

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Cleveland, from 40% in 1924 to 8.2% in 1935. He emphasizes, however that the change in Michigan had been "regardless of whether or not iodized salt had been used." His inferences regarding the decline in the percent of goiter in Michigan are terribly wrong. The marked decrease of the incidence of goiter was entirely due to the use of iodized salt. The data from which he quotes is from surveys which I made or directed.

Every article written on endemic goiter in Michigan giving the original data of these surveys, also stressed the deficiency of iodine in food and the use of iodized salt. In brief, Michigan has been the proving ground for the prophylaxis of endemic goiter by the use of iodized salt. From 1924 to 1932 more than 90% of the table salt used in Michigan was iodized. All information on the use of iodized salt was published in the same articles from which the data republished by Dr. Greenwald was taken.

It is difficult to understand how anyone could ever have read the literature on Endemic Goiter in Michigan and the use of iodized salt, and then have misunderstood it and misquoted it so completely as is done in this rewriting on the prevention of goiter in Michigan. I can only ask, "Why did he do it?"

His summary represents only personal opinions without scientific data or fair logic to warrant such conclusions.

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March 4, 1947

## LETTER TO THE EDITOR

### TO THE EDITOR:

Dr. Kimball objects to my corollaries. I shall discuss them in the order listed in my paper and his letter.

1. In his Harvey Lecture (p. 97), Marine classified simple goiter as a thyroid insufficiency and said that "simple goiter and myxedema . . . are but different stages or degrees of the same nutritional fault." I do not know why we should not draw the corollary that "Goitrous individuals should have low metabolic rates." The fact that they do not was not advanced by me as proof that goiter is not a thyroid insufficiency, for I can think of possible compensatory changes in other organs. Nevertheless, none of these possibilities has been demonstrated nor, so far as I know, even indicated by observations of any kind, clinical or experimental. I maintain my position.

2. "And again, there is no truth in the statement that goiters should contain less iodine than normal thyroid glands." May I quote from Dr. Kimball? (The Thyroid Gland, ed. Amy F. Rowland, Phila., 1922, page

168): "In other words, no functional hyperplasia and, therefore, no goiter can develop, at least in the mammals above mentioned (dog, sheep, ox, pig, rabbit, cat, and man. I.G.), if the iodine store in their thyroids is maintained above 0.1 percent." If, now, the iodine contents of goiters are as great as, or greater than, those of normal glands, and they are, we must assume that the increases in the weights of the glands more than compensates for the low concentrations. I regard such an assumption as entirely unwarranted. It seems to me to be quite obvious that if a goiter is due to a lack of iodine, the goiter should be found to contain less iodine than is present in a normal gland. I maintain that my corollary is logically derived from the proposition and that the fact that the observed facts are not in accord with the corollary is a severe blow to the proposition.

3. Immediately after quoting my corollaries, Dr. Kimball writes: "To even think that the above statements which he calls corollaries should be true shows a complete lack of understanding of simple goiter." Eight lines later, he asserts that Marine and his associates proved my corollary to be true! I do not know to which experiments, by Dr. Marine, Dr. Kimball refers. The only statement I can find is in the Harvey Lecture, pages 105 and 106. There is, however, no mention of iodine-free diets and the dose of iodine given was one milligram per week to each puppy. I submit that this dose cannot be regarded as merely making good a hypothetical deficiency and that the observations do not in any way substantiate the corollary. If Dr. Kimball knows of any experiments by Dr. Marine with iodine-free diets, I wish he would let me know where they were reported. I have not seen any reference to them in any paper by others who have attempted to produce goiter by lowering the iodine intake.

Dr. Kimball objects to my use of the data in his report of 1937. Just what the words "Instead . . . 1935" are intended to mean is not clear. They do not form a sentence. Perhaps some words have been omitted. Dr. Kimball also writes: "From 1924 to 1932 more than 90% of the table salt used in Michigan was iodized." I do not know just what the significance of this is supposed to be. According to Dr. Kimball's own summary of the Michigan census, there were a total of 61,649 children included in the survey. Of these, 9,429 are listed as *never* having used iodized salt. For the entire group of 61,649 children, whether they used iodized salt regularly, occasionally, or not at all, the reduction was, as stated, from 38.6% in 1924 to 8.2% in 1935. It is perfectly true that the Cleveland group that had not used iodized salt for three years showed no such reduction. I said so on page 732 and attempted an explanation on page 733. I have neither misunderstood nor misquoted the data.

The prophylactic use of iodine has not uniformly been followed by a reduction in the incidence of goiter. To the instances of *increases* in the oc-



currence of goiter cited in my paper, I would like to add those cited on page 193 of an article by Hercus and Purves (*Journal of Hygiene*, 26, 182-203, 1936). One observer reported: "In spite of the fairly general use of iodized salt, there appears from the year's figures to be a decided increase in the amount of goitre in all ages in both incipient and small classes." Hercus and Purves themselves observed several instances of goiter in women who used iodized salt (1:250,000) regularly. Dr. Kimball would, I supposed, retort that this amount is inadequate. Perhaps it is, but not because "one milligram per day, from iodized salt" (see his letter, page 2, line 27) is needed to make good a deficiency. A gram or more of quinine a day was once considered necessary to prevent malaria, but not because malaria was believed to be due to a lack of quinine.

"*Post hoc, ergo propter hoc*" is the oldest of the fallacies. The fact that toads appear after a rain is no proof that it rained toads. If Dr. Kimball will trouble to acquaint himself with the history of goiter, he will find that changes in the incidence of goiter as great, or greater than, any he has observed, have repeatedly been reported without any known change in the iodine intake and without the slightest reason for supposing that there was any such change.

In the last sentence of his first paragraph, Dr. Kimball writes: "These references suggest that some one is trying to bring this article to the fore. . . ." Perhaps they do suggest this to Dr. Kimball, but they will scarcely do this to anyone who approaches the discussion with an open mind. I wrote my article and you published it. *Science Service* found it of general interest. Miss Stafford telephoned to me for some additional information and prepared a release and a signed article. *Science Service* publishes hundreds of such releases every year. Newspapers print what the editors consider interesting. There is nothing mysterious about the procedure.

Dr. Kimball asks "Why did he do it?" There is only one answer. Because a careful examination of the literature led me to the conclusion that the "iodine-lack" hypothesis rested on no satisfactory evidence whatever; and was, in fact, contradicted by such evidence as is available. I have no apologies to offer, except to my students whom I had so long misled.

ISIDOR GREENWALD, M.D.  
477 First Avenue  
New York 16, N. Y.  
March 12, 1947

## LETTER FROM THE FORMER MANAGING EDITOR

TO THE EDITOR: —

This *Journal* has recently published an article written by Dr. Isidor

Greenwald, titled "Is endemic goiter due to a lack of iodine?" [6: 708-741 (1946)]. This work of Dr. Greenwald's has been the subject of an adverse Editorial in the *Journal of the American Medical Association* [133: 620 (1947)], and in view of the type of comments in that editorial and the fact that the decision for publication of Dr. Greenwald's article was made during my tenure of your present office, it seems appropriate, if you approve, to call to the attention of your readers the following:

When Dr. Greenwald submitted his article for publication the controversial nature of its contents was duly noted, and yet the several experts, whose advice was sought on the question of publication, were in agreement (with one exception) that this scientist deserved an opportunity to present his views on this subject. It is fair to say that while the opinions varied somewhat as to the emphasis that should be placed on the various parts, the current interest in antithyroid substances and the inconsistencies in the existing evidence, of which everyone was aware but which were considerably emphasized by Dr. Greenwald, had something to do with the decision to publish his article.

A lively reaction to the statements of Greenwald would be anticipated, especially by any one who attended the Third International Goitre Conference (1938), at which what seemed to be overwhelming evidence was presented supporting the iodine-lack hypothesis. Yet, even at that conference there was discussion of the information then available on antithyroid substances.

Too few students of goitre seem to be aware of the fact that nearly a century ago a commission was appointed by the French Academy to determine whether iodine deficiency was related to the causes of goitre. This Commission, after a careful study, reported in 1852 that they were unable to relate goitre to iodine deficiency because of inconsistencies in the data. This decision so discouraged scientific studies on the use of iodine in the prevention of goitre that more than fifty years had to pass before progress again could be made.

The Editorial in the *Journal of the American Medical Association* deals too abruptly with the matter, and with one sentence attempts to dispose of the author and his evidence. One wonders whether the writer of the Editorial read the original article as published in this *Journal*, for no reference was cited; there is merely a statement that "This extraordinary folly, unsubstantiated by anything resembling scientific evidence, has been circulated by *Science Service*."

We all naturally regret that the efforts of the Council on Foods and Nutrition, which has done so much good, may have been in any way embarrassed by this publication, and had any one connected with this been appraised of the state of affairs as presented in the Editorial, undoubtedly

publication could have been delayed. However, it would seem to be impossible for your Editors to prevent for long the publication of a sincere presentation of an opposing viewpoint by a competent scientist merely because an agency had once made a general decision on the subject.

Greenwald did not deny beneficial effects from iodide therapy, but stated in his summary, "The beneficial effects, such as they were, appear to have been due, not to the making good of a deficiency, but to a pharmacodynamic action of iodides." Should this conclusion of his be verified, iodide therapy would remain inescapable until some better method would be discovered.

Your readers will now understand that no matter what were the opinions of the individual editors and the guest referees of the *Journal of Clinical Endocrinology* on the relation of iodine to the cause of simple goitre, they believed that the subject deserved a presentation.

K. W. THOMPSON, M.D.  
(former Managing Editor)  
154 Upper Mountain Avenue  
Montclair, New Jersey  
March 13, 1947



# Announcements

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## SCHERING GRANTS TWELVE ENDOCRINE RESEARCH FUNDS

Mr. Francis C. Brown, president of Schering Corporation, of Bloomfield and Union, N. J., has announced that a substantial program of grants in support of medical research studies has been initiated by that corporation, manufacturers of endocrine, x-ray diagnostic, chemotherapeutic and pharmaceutical products.

The use of endocrine preparations in neuropsychiatry will be studied at the Worcester Foundation of Experimental Biology, Worcester, Mass., by Dr. Hudson Hoagland, and at the Montefiore Hospital's Division of Neuropsychiatry, New York City, under the direction of Dr. H. Houston Merritt, professor of clinical neurology at Columbia University, College of Physicians and Surgeons, and Dr. Arnold P. Friedman.

The value of hormones in treatment of internal bleeding and threatened abortion is to be investigated by Dr. Abraham Rakoff at Philadelphia's Jefferson Medical College and Hospital. Drs. Howard C. Taylor, Earl T. Engle and C. L. Buxton, professors of obstetrics, gynecology and anatomy, of Columbia University, College of Physicians and Surgeons, will study sterility in females, and early abortion. Investigation of the relationship of the adrenal gland to pregnancy and toxemias of pregnancy will be directed by Dr. Charles W. Lloyd, associate professor of medicine, at Syracuse University College of Medicine.

Three New York medical schools have been given grants to support studies on endocrines in the field of skin disorders in prepubescent youths, and to develop a new method of administration of estrogens. Dr. Frederick Reiss, associate professor of dermatology at New York University, College of Medicine, will plan the first program; the second at Columbia University's College of Physicians and Surgeons is under Dr. William A. Schonfeld; and the last will be directed by New York Medical College's professor of clinical medicine, Dr. Thomas R. McGavack.

Dr. Harrison F. Flippin, chairman of the committee on chemotherapy at Philadelphia General Hospital, will supervise the study of combined sulfonamide therapy in injections. Dr. Abraham Cohen, of the Philadelphia General and Jefferson Hospitals, will continue his study of the use of gold combined with estrogens and androgens in arthritis.

Dr. Walter M. Kearns, Milwaukee, Wis., continues for another year certain special research studies.

A large grant was given to the Massachusetts General Hospital, Boston, to support that institution's general research program.

### "ANDROGENS IN FEMALE" SUBJECT OF NEW 1947 SCHERING AWARD COMPETITION

"The Clinical Use of Androgens in the Female" has been selected as the subject for the 1947 "Schering Award" competition among medical students in the United States and Canada, according to Dr. John N. McDonnell, Director of Domestic Sales and Promotion for Schering Corporation, Bloomfield, N. J. As in previous years, cash prizes of \$500.00, \$300.00 and \$200.00 will be given for the best manuscripts received on this subject. Three judges prominent in endocrinology will select the winning entries. Dr. Norman L. Heminway, head of the Schering Medical Service Department, is chairman of the Schering Award Committee and in charge of the competition.

The "Schering Awards" are offered annually in competition to stimulate the acquisition of further knowledge of endocrinology by medical students, as a contribution to medicine by Schering, world's largest manufacturer of hormones.

Many students from almost a hundred medical colleges will submit their manuscripts for this year's Schering Award Contest which closes July 31, 1947. Even more widespread interest is anticipated in the 1947 "Schering Award" than in previous years.

### THE FOURTH INTERNATIONAL CANCER RESEARCH CONGRESS

The Fourth International Cancer Research Congress will be held in St. Louis, Missouri, U.S.A., September 2 to 7, 1947. The Union Internationale Contre le Cancer has accepted the invitation of the American Association for Cancer Research, and the congress will be held under the joint auspices of these two organizations, with Dr. E. V. Cowdry, Professor of Anatomy, Washington University School of Medicine, and Director of Research of the Barnard Free Skin and Cancer Hospital, serving as president of the congress.

Of the three congresses that have been held previously, the first was in Madrid, Spain, in 1933; the second in Brussels, Belgium, in 1936; the third in Atlantic City, New Jersey, U.S.A., in 1939. Due to the recent war, there has been no meeting of the congress during the past eight years.

The State Department in Washington has approved the International Cancer Research Congress, and official invitations soon will be sent to all foreign governments who are to send delegates.

Initial steps in the organization of the congress have been completed. All officers and committees have been appointed and are enthusiastically at work. In addition to the president, Dr. E. V. Cowdry, Dr. J. Godard, president of the Union Internationale Contre le Cancer, and Dr. W. U. Gardner, president of the American Association of Cancer Research, will serve *ex officio* as members of the executive committee.

The following committee personnel have accepted chairmanships:

- A. N. Arneson, St. Louis, Missouri—Local Arrangements
- S. Bayne-Jones, New Haven, Connecticut—Finance
- C. W. Larimore, New York, New York—Exhibits
- L. A. Scheele, Bethesda, Maryland—Governmental Liaison
- M. G. Seelig, St. Louis, Missouri—Publicity
- Shields Warren, Boston, Massachusetts—Program

Headquarters will be at the Hotel Jefferson, St. Louis, where some three hundred rooms will be available for guests. In addition to these rooms, other nearby St. Louis hotels have signified a willingness to make reservations on advance notification by those contemplating attendance at the Congress.



## Association Notice

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The 29th annual meeting of the Association for the Study of Internal Secretions will be held Friday and Saturday, June 6th and 7th, 1947, in the Viking Room of Haddon Hall Hotel, Atlantic City, New Jersey, preceding the Centennial meeting of the American Medical Association.

Members are urged to make reservations immediately inasmuch as the hotels expect to be filled to capacity. Make your reservations directly with Chalfonte-Haddon Hall advising them of the accommodations you wish. Rates are as follows:

	Chalfonte	Haddon Hall
Single room with bath:	\$6, \$7, \$9	\$7, \$8, \$10
Double room with bath (without ocean view):	\$8 and \$10	\$10 and \$12
Double room with bath (side ocean view):	\$12	\$14
Double room with bath (ocean front):	\$14 and \$16	\$16 and \$18

Make your reservations now and avoid disappointment—remember, you can always cancel them at a later date.

Further information regarding the meeting will be forthcoming at an early date.



# Abstracts of

## CURRENT ENDOCRINE LITERATURE

Editor; D. A. MCGINTY. Collaborators: A. R. ABARBANEL, F. N. ANDREWS, B. L. BAKER, F. A. DE LA BALZE, ISRAEL BRAM, R. A. CLEGHORN, RUCKER CLEVELAND, C. D. DAVIS, ANNA FORBES, M. B. GORDON, H. S. GUTERMAN, M. M. HOFFMAN, R. G. HOSKINS, C. D. KOCHAKIAN, H. S. KUPPERMAN, H. L. MASON, JANET W. MCARTHUR, THOMAS H. MCGAVACK, A. E. MEYER, K. E. PASCHKIS, A. B. PINTO, J. R. REFORZOMEMBRIVES, E. C. REIFENSTEIN, JR., G. G. RUDOLPH, L. T. SAMUELS.

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### ADRENALS

CONN, J. W., AND K. P. MATHEWS. Addison's disease in the Negro. *Am. J. Med. Sci.* 212: 404 (1946).

The authors report five cases of Addison's disease in Negroes and thus bring the total number of reported cases in Negroes to 26. They believe that there is undoubtedly a much greater number of recognized cases which have not been reported but that the largest group of cases of Addison's disease in Negroes is not being recognized as such. They believe that it is extremely doubtful that the incidence of Addison's disease in Negroes is smaller than in whites since analysis of their own cases leads to the opposite conclusion. They emphasize that increased pigmentation of the skin, which is the most striking hint to the physician of Addison's disease in whites, is of little or no value in suggesting this possibility to him as he examines the Negro patient. However, marked pigmentation on the dorsal surface of the tongue may be a significant finding. All of the cases of Addison's disease in Negroes reported in the literature upon which a pathological examination was made were found to be due to tuberculosis of the adrenals. One of the authors' cases was due to amyloidosis. From the information at hand, it would appear that idiopathic atrophy of the adrenal cortices (which now accounts for about 50% of all cases in whites) is either non-existent or extremely rare in the negro. The authors point out that this is probably not a valid conclusion. The authors conclude that physicians must be on guard for the recognition of Addison's disease in the Negro, which is in all probability much commoner than the number of reported cases would indicate.—*E.C.R., Jr.*

LEWIS, L. A., AND I. H. PAGE. Method of assaying steroids and adrenal extracts for protective action against toxic material (typhoid vaccine). *J. Lab. & Clin. Med.* 31(12): 1325-1329 (1946).

A method is described for determining the relative protective power of adrenal cortical extracts and keto-steroids against the toxic effects of typhoid vaccine in adrenalectomized rats. The substances to be tested is injected daily or twice daily (depending upon the menstruum) for three days prior to the intraperitoneal administration of 1.33 M.L.D. of typhoid vaccine. One protection unit has been defined as the amount of material per 24 hours which will protect 90 per cent of a group of adrenalectomized rats against the above mentioned dose of vaccine. By this method of assay, the relative potencies of several preparations in units per milligram were determined and found to be



as follows: 11-dehydro-17-hydroxycorticosterone, 5.5; 11-dehydrocorticosterone acetate (natural and synthetic), 3.6; 11-dehydrocorticosterone, 3.1; corticobenzate, 1.3; 11-ketoprogesterone, between 0.5 and 3.0; desoxycorticosterone, 0.5; progesterone, less than 0.3; and desoxycorticosterone acetate, acetoxyprogesterone, and methyl androstenediol, all less than 0.1. It is clear from the above that all of the compounds possessing an oxygen atom at C-11 exhibited considerable ability to protect animals from typhoid vaccine. The protective capacity of any given substance closely paralleled its activity as determined by the muscle-work test of Ingle. It seems likely, therefore, that a direct relationship may exist between the ability of these substances to protect against typhoid vaccine and their role in the regulation of carbohydrate metabolism.—*T.H.McG.*

SEGALOFF, A. The effect of diet on the growth and survival of adrenalectomized rats treated with desoxycorticosterone acetate pellets. *J. Lab. & Clin. Med.* 31(4): 482-483 (1946).

Immature, adrenalectomized male rats in which pellets of desoxycorticosterone acetate had been subcutaneously implanted were fed four variations of diet with the following results. No diet contained carbohydrate, but all had adequate types and amounts of vitamins for growth in the rat and U. S. P. XII salt mixture. Group I with crude casein and a fatty acid mixture in addition survived and grew during the experimental period of eight weeks. Group II without the fatty acid neither grew nor survived. Group III with a vitamin-free casein plus the fatty acid behaved similarly to group II. Group IV given a diet with fatty acid supplements but no casein acted as did group II. The authors concluded that dietary carbohydrate is not essential for the survival or growth of adrenalectomized rats treated with desoxycorticosterone acetate; that dietary fatty acids either burn as carbohydrate or permit such rats to utilize protein; and that crude casein contains a factor for growth not found in purified materials.—*T.H.McG.*

SHIPLEY, R. A., AND R. I. DORFMAN. The effect of infection and trauma on the excretion of urinary cortin. *J. Lab. & Clin. Med.* 31(4): 481-482 (1946).

The cold test of Selye and Schenker (modified) and the liver glycogen test of Dobriner et al. were used as qualitative methods of bio-assay for urinary cortin. Values for normal young men and women showed "no obvious differences," but the estimated amount of material present was greater for the cold test than for the glycogen test. Eighty patients with infectious diseases, two following the stress of operative procedure (herniorrhaphy), and three after extensive body burns were test subjects. The urines of all these showed a rise in the output of cortin as compared with the normal. During recovery, there was no evidence of an abnormal depression of cortin values.—*T.H.McG.*

SHIPLEY, R. A., R. I. DORFMAN, E. BUCHWALD, AND E. ROSS. The effect of infection and trauma on the excretion of urinary cortin. *J. Clin. Investigation* 25(5): 673-678 (1946).

Extracts of urine were tested for the presence of cortin-like material by (1) their ability to protect adrenalectomized rats from cold and (2) their capacity for causing the deposition of glycogen in the livers of mice. In both instances, the action was referred to standardized results achieved through the use of known quantities of 11-dehydrocorticosterone (Kendall's compound A). Forty-eight hour lots of urine from each of

17 normal persons between the ages of 24 and 38 years were thus studied, as well as similar specimens from 7 patients with infectious disease, 2 undergoing surgical procedures, and 3 with severe body burns. By the cold test, the normal subjects showed an average of 1.1 mg. of compound A-equivalent per day with a range of from 0.5 to 1.8 mg. By the glycogen test, the average was slightly less than 0.4 mg. of compound A-equivalent with a range of from less than 0.2 mg. to 0.8 mg. Sex made no apparent difference in these results. Five of the 7 patients with infectious disease had an increase in the excretion of cortin during the febrile and early post-febrile stages of their diseases; the other 2 were sick for only a day. Normal levels of excretion occurred throughout late convalescence. During the first 2 to 4 days following herniorrhaphy in each of 2 patients, there was a marked increase in urinary cortin to 10-fold the normal preoperative value (as measured by the glycogen deposition test) with a return to normal by the 6th postoperative day. In the 3 burned patients, distinct rises in urinary cortin occurred during the first 8 days; after the 12th day, the values were again within the range of normal. The authors emphasize the point that "during recovery from stress there was no abnormal depression in output which one would expect in the presence of adrenal exhaustion."—*T.H.McG.*

SPENCE, H. M. AND F. G. THOMPSON, JR. Hormone-producing tumor of adrenal cortex with congenital absence of contralateral adrenal gland. *New Eng. J. Med.* 236: 13 (1947).

A large adrenal tumor was removed to relieve a case of Cushing's disease. The patient went into adrenal crisis after the operation and died 36 hours after from adrenal insufficiency. On autopsy no adrenal gland could be found on the opposite side. The authors considered this as probably due to the depressing effect of the hormones produced by the tumor. The importance of pre- and post-operative therapy to offset the danger of the adrenal insufficiency is emphasized.—*L.T.S.*

WILLIAMSON, M. B. Concentration and properties of the adrenocorticotrophic substance in female human urine. *Proc. Soc. Exp. Biol. and Med.* 63(1): 191-194 (1946).

Freshly voided human female urine, dialyzed urine, and acetone precipitated urinary proteins were tested for adrenocorticotrophic activity in white rats by the methods of Moon, and Sayers, et al. for pituitary adrenocorticotrophins. Normal female urine contained adrenocorticotrophic activity not due to its estrogen content. The active material was nondialyzable and thermolabile and appeared to be a protein. It was similar to pituitary adrenocorticotrophin in its ability to produce adrenal weight increases and to depress the adrenal ascorbic acid level in normal and hypophysectomized male rats.—*F.N.A.*

## GENERAL

BARKER, H. B. A detailed report on the weights and weight losses of twenty-four men in Santo Tomas internment camp. *J. Lab. & Clin. Med.* 31(10): 1129-1132 (1946).

During 36 months the average weight loss for 1506 men was 51 pounds with a resultant weight which averaged 70 per cent of the prewar figure. For 1232 women, the loss was 32 pounds with a final weight which was 76 per cent of the value prior to war.

The greatest losses were sustained by the older men and women. Despite a diet yielding less than 1,000 calories and approximately 29 to 30 grams of protein daily, edema was quite uncommon. Although details are not given, the author states that pulse rates were usually slow; and that blood pressures of more than 100 mm. of mercury, and red cell counts of over 4 0 million per cu mm. were exceptional findings among the camp population.—*T.H.McG.*

BRADBURY, J. T. A simplified method for the estimation of sodium. *J. Lab. & Clin. Med.* 31(11):1257-1261 (1946).

A simplification of the uranyl-zinc method for the determination of sodium in body fluids is described. The original method is based upon the ability of uranyl-zinc acetate to precipitate sodium as a triple salt. When redissolved this triple salt gives a colorimetric reaction directly proportional to the number of uranyl ions present in the solution. The author's modification consists in estimating the loss of such color in the supernatant fluid, and from this calculating the amount of sodium necessary to cause such a withdrawal of uranyl. The method checks well with gravimetric determinations. The author states that "The method can be used when sodium chloride concentrations are as low as 0.5 mg. per cubic centimeter."—*T.H.McG.*

CARR, E. A. A rapid bedside test for the detection of hypoglycemia. *J. Lab. & Clin. Med.* 31(11):1267-1269 (1946).

This method of determining hypoglycemia depends upon an adaptation of the oxidized bismuth-alkali method of detecting sugar in various bodily fluids. Whole blood is precipitated with copper sulfate and filtered under pressure. A drop of the filtrate is allowed to fall upon a large excess of Galatest (or other similar) powder. If within 45 seconds a definite grey or black discoloration develops, the reaction is considered normal. If such a change in color fails to appear, then hypoglycemia is present. In actual tests, a positive grey or black discoloration occurred in all blood sera containing 53 or more milligrams of glucose per 100 cc. Abnormal reactions were observed in all blood specimens containing 44 mg. per 100 cc. or less. All reagents are stable but the powder must be kept dry. The test is designed to consume not more than 3.5 minutes of time and can be carried out at the bedside.—*T.H.McG.*

CHESNER, CHARLES. Hemochromatosis: Review of literature and presentation of a case without pigmentation or diabetes. *J. Lab. & Clin. Med.* 31(9):1029-1036 (1946).

An atypical case of hemochromatosis occurred in a 14 year old boy who had been anemic for 6 years and had complained of progressive weakness and fatigue for 6 months prior to admission to the hospital for an upper respiratory infection. A moderately severe, hypochromic, microcytic anemia and a very large spleen were found. The latter was removed. Enlargement of the liver was noted for the first time some 5 months later when he was admitted to the hospital for the third time because of weakness, pallor and severe anemia. Two and one-half months later, he was again admitted for abdominal pain, weakness, fever and exacerbation of the anemia. Clinically the "diagnosis varied from subacute bacterial endocarditis to acute surgical abdomen secondary to diseased gallbladder or appendix." Following postmortem examination, the diagnoses were: "Hemochromatosis with hemosiderosis of the liver and pancreas and generalized hemosiderosis; portal cirrhosis of the liver; recent thrombosis of portal and superior

mesenteric veins with hemorrhagic infarction of the lower three-fourths of the small intestine; atelectasis of the right lower lobe; Ghon tubercle in the lower lobe of the right lung; absence of spleen (surgical removal)." The striking and atypical features of his hemochromatosis included his youth; the complete absence of diabetes and of abnormal pigmentation; the presence of anemia for at least 6 years before other disturbances were noted; and the unusual manner of death with portal and mesenteric thrombosis. The diagnosis was based upon the finding of pigment cirrhosis of the liver and pancreas and siderosis in other organs. Several theories regarding the pathogenesis of hemochromatosis are discussed. The authors do not believe repeated transfusions played any part in their case as a total of 2.75 gm. of iron via transfusion were given to the patient over a period of 9 months whereas at autopsy 47 grams of iron was recovered from the liver alone—*T.H. McG.*

ELLIOTT, J. E. AND P. B. PEARSON. A direct photoelectric method for the determination of serum calcium. *J. Lab. & Clin. Med.* 31(11): 1262-1266 (1946).

The method for the determination of serum calcium is based upon the precipitation of calcium as the oxalate and the photoelectric or spectrophotometric measurement of the amount of potassium permanganate reduced by using an excess of this reagent. The values obtained agree well with those derived by electrometric titration with standard permanganate solution.—*T.H. McG.*

HORVATH, S. M., H. GOLDEN, AND J. WAGER. Some observations on men sitting quietly in extreme cold. *J. Clin. Investigation* 25(5): 709-716 (1946).

Forty-five men with an average age of 20.5 years, and "in excellent physical condition" were subjected to a total of 430 tests at temperatures varying from 1.1° to -40° C. Metabolic rates, skin and rectal temperatures and subjective phenomena were continuously recorded over a period of three hours and compared with control values obtained with the ambient temperature at 22° C. "The heat production in the cold was above basal values during the entire test period. In the -40° C. environment average metabolic increases of 13, 53, and 74 per cent were recorded for the first, second and third hours respectively. The rise in heat output during the first hour could not be explained on the basis of shivering. In the third hour, shivering was present in the majority of the subjects. Neither the role of chemical mediators, nor that of increased muscular tonus, could be clearly delineated, and require additional investigation. The fall in rectal temperatures was moderate, although values of 35.4° C. were occasionally observed. The absolute value was not correlated with the presence of shivering and, therefore, low rectal temperatures could not be considered as the stimulus for shivering." The rectal temperature continued to fall for some time after the subjects were returned to an environmental temperature of 22° C. The greatest fall in the mean skin temperatures (an averaged figure for each individual taken from readings on the thigh, toe, arm, calf, and chest) was observed in the first hour of exposure to cold. However, there was considerable variability in the control figures, and in different subjects during any single test as well as in the same subject on repeated test. At the end of 3 hours the average mean skin temperature with an ambient temperature of -17.8° C. was 27.2° C.; and with a surrounding temperature of -40° C. it was 24.5° C. As would be expected, the decrease in the temperature of the toes was greater than for the other parts of the body,

temperatures below 0° C. being recorded in environments of -23° C. or below—*T.H.McG.*

KESMODEL, K. F. Carcinoma of the breast. *South. Med. Jour.* 40(1): 43-46 (1947).

In a discussion of the palliative measures or therapy that may be used in incurable carcinoma of the breast, testosterone propionate was mentioned as an aid in alleviating the pain. The dose used must be large (3,975 mg. in three months in one patient). The author advised frequent blood chemical studies with particular attention paid to the serum calcium level when large doses of testosterone propionate were used for long periods of time.—*H.S.K.*

LEVINE, E. B., AND A. L. SELLERS. Testosterone in angina pectoris. *Am. J. Med. Sci.* 212: 7 (1946).

The authors have reviewed the reports in the literature on the treatment of angina pectoris with testosterone compounds and have reported their experience with this therapy in 24 male patients referred to them with the provisional diagnosis of angina pectoris. They conclude: 1) Neither testosterone propionate intramuscularly nor methyl testosterone sublingually appears to have any value in the treatment of angina pectoris. 2) Testosterone preparations appear to be of definite value in relieving the chest discomfort sometimes associated with the male climacterium or the similar precordial ache of neurocirculatory asthenia occasionally encountered in individuals in the age group commonly subject to angina pectoris. 3) In their field of usefulness, parenteral administration of 25 mg. of testosterone propionate two or preferably three times weekly is to be preferred to the rather ineffective administration of methyl testosterone sublingually in doses of 10 or 15 mg. daily.—*E.C.R., Jr.*

SHARPEY-SHAFER, E. P. 2-Thiouracil in the treatment of congestive heart failure. *Brit. Med. J.* 2: 888 (1946).

Data are given on 12 cases of congestive heart failure treated with thiouracil to produce hypothyroidism and decreased oxygen consumption. Clinical effects on both low-output heart failure and heart failure with emphysema were good. Along with the decreased oxygen consumption and development of clinical evidences of myxedema, there occurred a decrease in the venous arterial oxygen difference, particularly in the low output group. The work of the heart was not changed in some cases although clinical benefit was observed.—*L.T.S.*

## PITUITARY

MILLER, R. A. Pituitary hypothyroidism with impaired renal function. *Brit. Med. J.*: 650 (1946).

A case is reported in which a shrapnel wound in the frontotemporal region caused loss of weight, hypothyroidism, impairment of renal function, and a reversal of the concentration of the day and night urine. These symptoms were interpreted as being the result of pituitary damage, even though there was no permanent cachexia, hypogonadism, or lowering of the blood sugar.—*L.T.S.*

SPAIN, A. W. AND GEOGHEGAN, F. Diabetes insipidus with postpartum pituitary necrosis. A report of two cases. *J. Obst. & Gynaec. Brit. Emp.* 53 (3): 223 (1946).

Collapse, usually associated with hemorrhage, at childbirth is relatively frequently followed by necrosis of the anterior pituitary. The authors report two such cases in which polyuria preceded death which occurred on the tenth and fourth day postpartum, respectively. Extensive degenerative lesions were found in the posterior lobe of the pituitary in each case. In both, some anterior lobe cells had survived.—R.A.C.

YOUNG, F. G. Growth and diabetes in normal animals treated with pituitary (anterior lobe) diabetogenic extract. *Biochem. J.* 39: 515-536 (1945).

Normal dogs, cats, and rats were fed a meat diet just sufficient to maintain a constant body weight under normal circumstances. Daily injections of diabetogenic anterior pituitary extract led to nitrogen retention, a rise in body weight and, in the dog and cat, glycosuria. Analysis of the tissues indicated that protein had been deposited at the expense of fat. The loss of calories entailed by formation of new tissue and glycosuria was more than balanced by an increased oxidation of stored fat. An important action of the extract appeared to be the partial replacement of carbohydrate and protein combustion by that of fat. An equicaloric balance between the oxidation of extra depot fat and the deposition of tissue having the composition of muscle would lead to a substantial increase in body weight. Such an effect accounts for the observed increase in weight. A decrease in fat content and a gain in protein-containing tissue result in a higher mean specific gravity. It is suggested that a knowledge of the mean specific gravity might be helpful in cases of pathologic overweight. It is suggested as probable that diabetes represents the pathologic result of an excessive stimulation of those processes (inhibition of carbohydrate oxidation with enhancement of protein storage and of fat combustion) which normally lead to such deposition of new tissue as is associated with growth.—H.L.M.

## THYROID

BALZE, F. A. DE LA. Influencia del hipertiroidismo sobre la densidad y contenido en proteínas del plasma. *Medicina* 6: 347 (1946).

The author studied the specific gravity and the protein content of the blood plasma in 13 cases of thyrotoxicosis before and after surgery. Postoperatively there was a slow but constant increase in the specific gravity. There was also a rise in the protein content of the plasma which was most pronounced in the most severe cases. The change in protein content was less closely co-ordinated with the postoperative increase in weight, and was not related to the increase in basal metabolic rate after operation. Hemoconcentration seemed to be one of the factors responsible for the increased plasma density. The author suggests that these changes may be etiologic factors in the ophthalmic disturbances of hyperthyroidism.—F. A. de la B.

BOROVSKI, M. L. The role of the nervous system in autotransplantation of the thyroid. *Arkhi. Pat. Anatomii i. Pat. Fizeologii* 7 (2): (1941).

The nerve supply of the normal thyroid gland in rats, rabbits, and guinea pigs consists of a complicated nerve network without endings encircling the blood vessels of the interstitial tissue and penetrating the interfollicular spaces. Autotransplantation of

glands in rats is most successful from 1 to 14 days of age, due to the more powerful regenerative capacities of the organism, particularly the nervous system, during this period. Histologically, no change was seen in the nerve fibers of the transplanted gland.

Regeneration of the fibers in transplants begins on the 15th day and is complete by the 45th day. In cases where the transplant does not attach, absorption occurs between the 19th and 21st days. However, if it was attached within 45 days, absorption was never observed. If the gland did not attach, there was a rapid proliferation of new nerve fibers simultaneously with degeneration of the original nerves. The nerve network of the transplant during the absorption period shows vitality and decays partly during and partly after the decay of the parenchyma. This can be explained only on the basis that the nervous system controls degeneration as well as regeneration of the transplanted gland. This work is, according to the author, the first demonstration of nerve fibers in the transplanted thyroid of rats, rabbits, and guinea pigs, and he concludes that the attachment or absorption of the thyroid gland after transplantation is organized and regulated by the nervous system.—Courtesy J. H. *American Review of Soviet Medicine*.

BRIGGS, G. M. AND LILLIE, R. J. Perosis caused by feeding high levels of thiouracil. *Proc. Soc. Exp. Biol. and Med.* 61 (4): 430-432 (1946).

Perosis is a common deformity of the leg bones of growing chickens and is known to have been caused by dietary deficiencies of manganese, choline, biotin, nicotinic acid, and perhaps some unidentified factors. A total of 91 day-old chicks were fed a standard ration or the same ration to which thiouracil was added at a level of 0.5 per cent for a period of five weeks. Forty-six of 47 chicks receiving thiouracil developed perosis, as characterized by an enlargement of the tibiometatarsal joint and by a thickening and bending of the metatarsals. The supplementation of the thiouracil-containing ration with manganese, choline, nicotinic acid, biotin, or riboflavin did not prevent the condition. It was suggested that thyroxin may, in some manner, aid in the prevention of perosis under normal conditions.—*F.N.A.*

BURGESS, A. M. Myxedema controlled by thyroid extract for fifty-two years: report of a case. *Ann. Int. Med.* 25: 146-150 (1946).

The clinical history and autopsy findings are presented of a patient who developed myxedema at the age of 35, was put on thyroid at the age of 39 and died when almost 92 years old. This is probably the longest period of successful treatment of myxedema on record. It is especially remarkable in view of the fact that during the last 20 years the patient suffered from hypertensive and arteriosclerotic heart disease.—*J.M.*

CASTILLO, E. B. DEL, GALLI MAININI, C., FINOCHIETTO, R., LUCHETTI, S. E. AND STAFFIERI, J. J. El tiouracilo como tratamiento preoperatorio del hipertiroidismo en 37 pacientes. *Medicina* 6: 221 (1946).

The authors describe their experiences with thiouracil as preoperative treatment in 37 cases (33 women and 4 men) of thyrotoxicosis.—*F. A. de la B.*

FOLDES, F. F. AND MURPHY, ANNA J. Distribution of cholesterol, cholesterol esters and phospholipid phosphorus in blood in thyroid disease. *Proc. Soc. Exp. Biol. and Med.* 62 (2): 218-223 (1946).

The distribution of total cholesterol, cholesterol esters, and phospholipid phosphorus was studied in the blood cells and plasma of 7 hypothyroid and 12 hyper-

thyroid humans. Plasma cholesterol, plasma cholesterol ester, and plasma phospholipid phosphorus were significantly increased in hypothyroid patients and only plasma phospholipid phosphorus was significantly decreased in hyperthyroidism. The plasma cholesterol ester / total cholesterol ratio and the plasma cholesterol / plasma phospholipid phosphorus ratio were significantly increased in hypothyroidism and the cell cholesterol / plasma cholesterol, and the cell phospholipid phosphorus / plasma phospholipid phosphorus ratio were significantly decreased. The only significant change in hyperthyroidism was the increase of the cell phospholipid phosphorus / plasma phospholipid phosphorus ratio. The lipid values and the various lipid ratios of both hyper- and hypothyroid patients returned towards normal following adequate treatment.—*F.N.A.*

JACKSON, A. S. Thiouracil will not replace thyroidectomy. *Surg., Gyn. and Obst.* 83: 249-252 (1946).

Thiouracil will supplement, but not supplant, thyroidectomy in the treatment of toxic goiter, in the author's opinion. He regards thiouracil therapy as contraindicated in the management of toxic adenoma, since adenomas are neoplasms, and the patient may later succumb to malignancy or intrathoracic goiter. He deplores the necessity for prolonged treatment with a toxic drug whose capacity to induce long-term remissions is as yet undetermined. Types of cases in which thiouracil makes a distinct contribution to management are: (1) advanced cases of multiple toxic adenoma in which there is no response to iodine; (2) cases of toxic adenoma complicated by such conditions as myocarditis, fibrillation, hypertension, diabetes and decompensation; (3) cases of severe exophthalmic goiter in the very young, the aged, the debilitated, and the decompensated; (4) cases of exophthalmic goiter in which, because of pregnancy, previous nerve injury, infections or other diseases, it may be desirable to delay surgery and yet not continue with iodine; (5) cases of iodine-fast exophthalmic goiter; (6) cases of persistent or recurrent hyperthyroidism.—*J.M.*

LERMAN, J., JONES, H. W. AND CALKINS, E. Studies on two sporadic cretinous brothers with goiter, together with some remarks on the relation of hyperplasia to neoplasia. *Ann. Int. Med.* 25: 677-701 (1946).

Two patients with sporadic cretinism and goiter are presented. The thyroid glands removed at operation were similar in appearance microscopically and showed areas of fetal adenoma, papillary cystadenoma, struma nodosa micro et macrofolliculare, and involution. The similarity of these glands to the thyroids of cretinous pigs whose parents were fed on a soybean diet and to the thyroids of certain patients given thiouracil preoperatively is pointed out. The fragmentary evidence suggesting that neoplasia may be the end-result of extensive hyperplasia is discussed.—*J.M.*

LINNELL, J. W., KEYNES, GEOFFREY AND PIERCY, J. E. Some vulgar errors in regard to goiter. *Brit. Med. J.*: 449 (1946).

The article is a plea for the early surgical treatment of goiter even though it is not patently toxic. The authors discuss 11 errors which they feel are commonly made by many medical men. The first is that apparently nontoxic goiters are harmless. The next three points attacked are the ideas that tachycardia, loss of weight, and elevated basal metabolic rate must be present for the diagnosis of toxic goiter. Under the next three headings the authors attack the ideas that iodine, x-ray, or thiouracil are adequate curative treatments for goiter. The next three points attacked are various objections raised



to surgical removal. The last point which the authors make is that thyroid surgery requires experience. The discussion is general and does not deal with specific data.—*L.T.S.*

MEANS, J. H. Evaluation of the several methods for treating Graves' disease available today. *Ann. Int. Med.* 25: 403-411 (1946).

The relative merits of (1) subtotal ablation of the thyroid, (2) medical treatment with antithyroid drugs, and (3) irradiation of the thyroid by means of radioactive iodine, for the treatment of Graves' disease, are evaluated. The author favors thyroidectomy after preparation with an antithyroid drug in combination with iodine, over prolonged use of an antithyroid drug alone. Judgment regarding the ultimate place of radioactive iodine therapy is withheld pending more extensive and prolonged utilization.—*J.M.*

TRASOFF, A., WOHL, M. G. AND MINTZ, S. S. Fatal agranulocytosis with autopsy following use of thiouracil in case of thyrotoxicosis. *Am. J. M. Sc.* 211: 62 (1946).

The authors report a fatal case of agranulocytosis in a 34 year old man who had been treated for seven months with thiouracil (0.6 to 0.1 gram daily) for thyrotoxicosis. The patient developed acute membranous pharyngitis, meningeal irritation, and succumbed after an illness of three days. At autopsy, the thyroid showed histologically focal hyperplasia, and focal colloid goiter (iodine had been administered), and the bone marrow was hypoplastic. The patient exhibited a high degree of pyrexia, marked irritability, restlessness, and hyperkinesism during the entire course of his hospital stay, which required shackling to restrain him even after the use of morphine and other sedatives. The authors interpret these findings as evidence of an aggravated state of thyrotoxicosis resulting from the infection and suggest that a thyroid crisis contributed to the fatal termination.—*E.C.R. Jr.*



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## EXCRETION OF GLYCOGENIC CORTICOIDS AND OF 17-KETOSTEROIDS IN VARIOUS ENDOCRINE AND OTHER DISORDERS

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THERE have been a number of methods of estimating the function of the adrenal cortex in clinical disorders. First has been the observation of clinical symptoms and signs and the grouping of these into the classical syndromes associated with gross diseases of the adrenal cortex. Difficulties have arisen in that many cases do not possess all the features of the well established disease and terms such as Addisonism and hypoadrenalism have been coined to describe those clinical states in which the evidence for hypofunction of the adrenal cortex is often largely inferential. On the hyperfunctional side the association of virilism with hyperplasia and tumors of the adrenal cortex has resulted in the tendency to attribute all cases of simple hirsutism to hyperfunction of this gland.

Second, the observation at autopsy or at operation of gross and microscopic morphological changes in the gland has been used. It was by this method that the classical syndromes were established. The method cannot of itself, however, detect changes in function which may occur without gross morphological change and the observation in clinical cases is usually a single one often made after the death of the patient. Also the association of changes in lipid content directly with functional change cannot be

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done on morphological grounds alone; for example, a depletion of the lipids of the gland does not necessarily indicate functional exhaustion.

Third has been the use of various metabolic indications such as disturbances of electrolyte and carbohydrate metabolism, which have been shown experimentally to be influenced by adrenal cortical function. These are indirect measurements which may be affected by other glands and other factors than the function of the adrenal cortex.

Fourth has been the measurement in blood or urine of the compounds secreted by the adrenal cortex or of their metabolic products. The so-called total neutral 17-ketosteroids have been used for this purpose and have been shown to be low in cases of Addison's disease and high in cases of adrenal tumor. They have also been extensively used to indicate changes in adrenal function of a lesser degree. This determination suffers from the disadvantage of all measurements of excretion products in that a number of factors beside the actual rate of secretion by the gland affect their rate of excretion. 17-ketosteroids are derived not only from the adrenal cortex but, in the male, from the testis. The fact that there are a large number of them and that changes in individual members of the group may occur and have significance apart from the total rate of excretion has been well shown by Dobriner (2) and his group.

Fifth the measurement of biologically active corticoids. This has been done in the adrenalectomized animal, either by life maintenance, protection against stress, or by measurement of the activity of these substances in carbohydrate metabolism. This method suffers also from the disadvantage mentioned above in that the relation of secretion of the gland to level of excretion of the material measured, may be affected by many factors. So far as is known, highly active substances of this type are derived only from the adrenal cortex.

Finally, chemical methods involving colorimetric procedures have been devised for assaying adrenal cortical steroids. The methods of Talbot and of Heard and Sobel are based upon the reducing properties of adrenal steroids, that of Lowenstein, Corcoran and Page, on periodate oxidation of the primary alcohol group at  $C_{21}$  which yields formaldehyde which can be measured colorimetrically. These methods may include metabolites of adrenal hormones which are not active in the biological assay and give much higher values than the bioassay.

It has become apparent in recent years through the work of investigators that the adrenal cortex has a variety of functions. The substances so far derived from it may be roughly divided into three groups: (a) the compounds which act mainly on electrolyte metabolism, (b) the compounds which act mainly on protein and carbohydrate metabolism, (c) a group

which has properties of the sex hormones. In this group fall the androgens which also affect protein metabolism, the estrogens and progesterone. An increased or decreased secretion of these substances might then lead to qualitatively different states of hyper or hypo function of the adrenal depending upon the relative amounts of the various substances secreted. The qualitative and quantitative variations in function thus produced could lead to a variety of metabolic and clinical disorders some of which may not have been previously associated with disturbances of adrenal function, and others of which account for the variation in the clinical and metabolic picture in cases grouped under the headings of Addison's disease, Cushing's syndrome, adreno-genital syndrome, etc. Qualitative variations in cases falling into the category of Cushing's syndrome and the possible relationship of these to variations in adrenal function, have recently been discussed by Kepler and Sprague (8).

It seemed of interest to apply a recently developed biological method of determining adrenal steroids possessing activity in carbohydrate metabolism to the determination of urinary corticoids in various clinical disturbances and to compare the variations in this type of activity with the variations in total 17-ketosteroids. One has in this biological assay a method of measuring, even though indirectly, one type of adrenal cortical function. Neither the group of substances purely active in electrolyte metabolism nor the androgens, etc., affect the assay in the quantities in which they are likely to be found in the urinary extract used. The biological method developed by Venning, Kazmin and Bell (12), based upon the ability of adrenal substances to cause a deposition of glycogen in the liver of the adrenalectomized fasted mouse, was used in these studies.

It has previously been shown by several authors that there are present in normal human urine substances that have the properties of adrenal cortical compounds. Anderson, Haymaker and Joseph (1), reported that extracts from the blood and urine of patients with Cushing's syndrome were effective in prolonging the life of adrenalectomized rats. Weil and Browne (14), found an increased excretion of substances active in the Selye-Schenker cold exposure test in the urine of a patient suffering from Cushing's syndrome, in some cases of hypertension, as well as after injury, infections and surgical operations. Dorfmann, Horwitt and Shipley, (3) were unable to detect cortin-like material using a cold exposure test in fourteen out of fifteen samples of urine from seven patients with Addison's disease, whereas urine from normal individuals did show activity in this test. The excretion of 17-ketosteroids and glyco-genic corticoids in normal individuals has been discussed in a previous paper (13). The present one deals with the findings in various endocrine disorders. Preliminary reports

of our investigations have been presented at the Conferences on Metabolic Aspects of Convalescence, 1942-46, held under the auspices of the Josiah Macy, Jr., Foundation.

### METHODS

A 48-hour specimen of urine was collected on successive days. No preservative was used but the urine was kept cold after collection. The volume was measured, 100 cc. removed for the 17-ketosteroid determination, and the remainder was acidified to pH 1.0, extracted and assayed for glycogenic corticoids according to the method of Venning, Kazmin and Bell (12). The activity is expressed in terms of glycogenic units excreted per twenty-four hours, one glycogenic unit being equivalent to the biological activity contained in one microgram of 17 hydroxy-11 dehydro corticosterone. Colorimetric measurements of the 17-ketosteroids were performed according to the method of Holtorff and Koch (7), and a correction factor for non-steroidal chromogens was applied.

### RESULTS AND DISCUSSION

#### *Normal Individuals*

These values have been reported previously (13) but are listed in Table 1 for comparison with the results in abnormal conditions. The excretion of both 17-ketosteroids and of glycogenic corticoids is found to be higher in the male than in the female adult. While little or no glycogenic activity could be detected in the urine of the new-born male infant, by the age of 2½ years there was already present an amount within the range found in the urine of the normal adult. The values for 17-ketosteroids and for glyco-

TABLE 1. EXCRETION OF 17-KETOSTEROIDS AND GLYCOGENIC CORTICOIDS IN THE NORMAL INDIVIDUAL

Sex	No. cases	Age	17-Ketosteroids		Corticoids	
			range	average	range	average
		years	mg.	mg.	gl. units	gl. units
Female	12	21-48	7-18	11	25-55	39
Female	2	74-76	6-8	—	26-38	—
Male	12	20-51	10-25	17	40-85	60
Male	2	77-81	10	—	20-37	—
Male	6	1-4	—	—	0-15	—
		days				
Male	2	3	2	—	40-42	—
Male	1	5½	5	—	53	—
Male	1	12	6	—	55	—

genic corticoids did not necessarily parallel each other even in adults. In the case of young children the corticoids reached adult levels long before the ketosteroids. It has also been reported by Venning (11) that in late

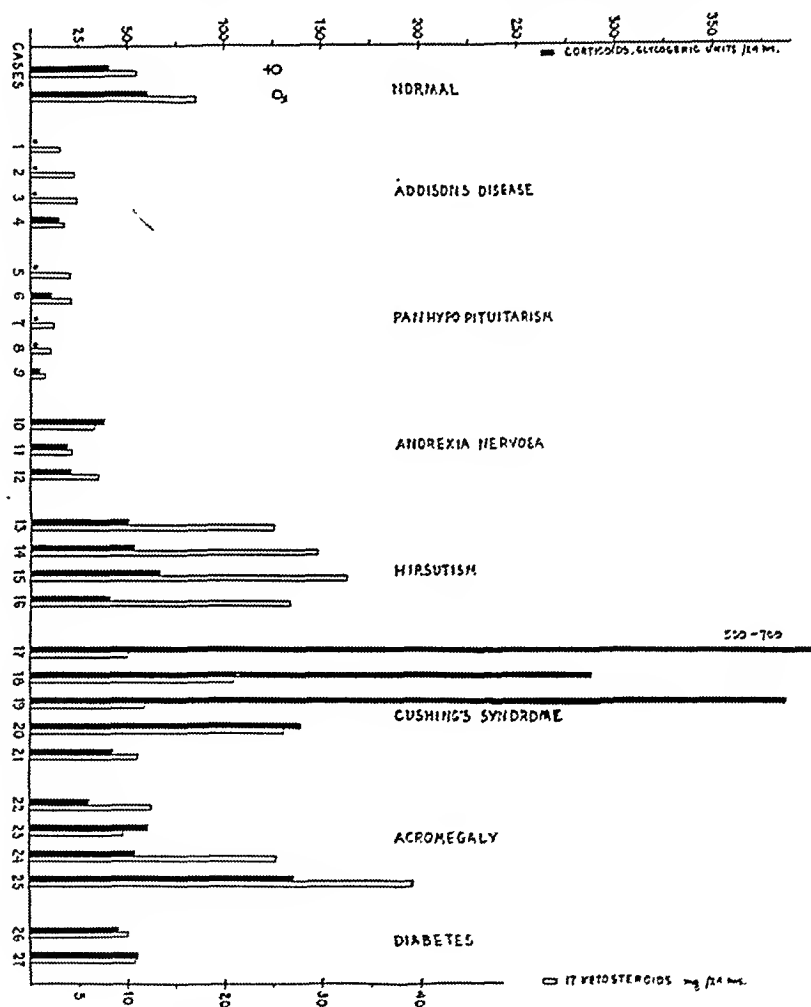


FIG. 1. Excretion of Glycogenic Corticoids and 17-Ketosteroids in Various Endocrine Disorders.

pregnancy the glycogenic corticoids reach very high values whereas the true 17-ketosteroids do not rise. It must be borne in mind that the 17-ketosteroids are excreted in very much larger amounts than the corticoids, milligrams of 17-ketosteroids as against micrograms of corticoids. The corticoids are labile substances and their degree of destruction or conversion into other substances in vivo is unknown; the excretion may repre-

sent only a small fraction of the total amount elaborated in the gland itself. This, however, still permits interpretation in terms of relative changes from the normal level in each.

### *Pathological Conditions*

The patients were divided into eight groups and brief descriptive histories and the results of the assays are presented in Tables 2 to 9. The excretion of corticoids and 17-ketosteroids in the various clinical disorders are shown graphically in Fig. 1.

### I. Addison's Disease (Table 2)

Four cases were studied, one male and three females, all showing typical symptoms and signs. In all four cases the 17-ketosteroids were low. In

TABLE 2. ADDISON'S DISEASE

Case No.	Clinical data	Date	17-Ketosteroids	Corticoids
1	W.—Male, 28 hr., fatigue, weakness, nausea for 2 yr., abdominal pain, characteristic pigmentation, no calcification adrenals, no tuberculosis, maintained with D.C.A. pellets.	1944	mg./24 hr. 2.3 3.8	Gl.U./24 hr. <10 —
2	B.—Female, 56 yr., weakness, nausea 2 yr., pigmentation on skin and mucous membranes, loss of weight, no pubic or axillary hair, pulmonary tbc in past, admitted in Addisonian crisis. B.P. 80/40, serum Na 130 m.eq./l, serum K 6.8 m.eq./l. At the time of assays (1) and (2) pt. was receiving 5 cc. adrenal cortical extract and 5 mg. D.C.A. Assays covered a period of 20 days.	1945 (1) (2) (3) (4) (5) (6)	2.5 6.8 4.3 6.2	— 15 — <10 <10 <10
3	McK.—Female, 29 yr., fatigue, anorexia, vomiting, gradual pigmentation and amenorrhea 7 mos., increased glucose tolerance, insulin sensitivity test showed failure of B.S. to rise, B.P. 90/60, serum Na 134 m.eq./l. Maintained on D.C.A.	1944  1945	4.5 4.4 — 5.2	<10 <10 <10 <10
4	D.L.—Female, 39 yr., headache, nausea, weakness, fatigue, pigmentation 2 yr., anorexia, loss of weight, tbc. hip at age 7, no calcification of adrenals. Serum Na 130 m.eq./l, serum K 5.3 m.eq./l. B.P. 98/66.	1945 May June Nov.	1.9 4.4	23 10 22

cases 1 and 3 (patient of Dr. E. H. Mason), less than the amount of glycogenic corticoid activity detectable by the method was present. In case 2, in the second assay, 15 glycogenic units were detected, the patient was receiving daily at this time 5 cc. of adrenal cortical extract intramuscularly and it is possible that some of the active material from this was excreted in the urine. In case 4 the amounts varied from below normal to low normal on various occasions. This patient was maintained on desoxycorticosterone sublingually 5 to 7 mg. daily, and on occasions received intramuscularly, an additional 5 to 10 mg. She had marked weakness and definite brown pigmentation. Her serum sodium which was consistently low, remaining about 135 m.eq./l, was difficult to raise to normal with DCA therapy. On one occasion, when the dose of DCA was reduced, it fell as low as 124 m.eq./l. She responded best clinically to a combination of DCA and cortical extract. She menstruated regularly and showed no signs of virilism. She had never had signs of hypoglycemia. That it is possible to have hypofunction of one aspect of adrenal metabolism and normal or hyperfunction of the others is seen in two cases reported recently. Wilkins, Fleischmann and Howard (15), reported a case of a 3½ year old boy who had precocious development of the male accessory sex organs accompanied by pigmentation of the skin and gums, low blood sodium and high NPN. Talbot (10) described a case of a child with the electrolyte disturbances and pigmentation of Addison's disease, with normal oxysteroids as determined by his chemical procedure. This patient also had premature sexual maturity and markedly raised 17-ketosteroids. Talbot has suggested a division of Addison's disease into those cases with and without hypoglycemic crises. It is questionable whether these are qualitatively different types of disease or represent stages in the process of destruction of the gland. In connection with Addison's disease one may ask at what point in the diminution of adrenal function do the various symptoms and metabolic changes appear. It is possible that one type of function of the adrenal may decrease more rapidly than another or independently of the other functions. We have at present unfortunately no method of measuring the purely electrolyte regulating function of the adrenal by means of any excretion products.

The present findings, together with those of Dorfman, Horwitt and Shipley (3) and cases cited by Forbes (5), indicate that the biologically active corticoids are very low in the majority of cases of Addison's disease. Further cases will have to be studied to determine whether there are variations dependent upon the stage or severity of the syndrome, or whether within the syndrome qualitative variations in the type of adrenal deficiency can be detected.



TABLE 3. PANHYPOPITUITARISM

Case No.	Clinical data	Date	17-Keto-steroids	Corticoids
5	B.—Male, 48 yr., fatigue, pallor, skin dry and thin, absence of facial, pubic and axillary hair, impotence, testicular atrophy, headache, craniopharyngioma removed in 1924. B.M.R. -25%.	1945	mg./24 hr. 3.7 4.5 3.6	Gl.U./24 hr. <10 <10 <10
6	T.—Male, 32 yr., well nourished, hypersensitivity to cold, fatigue, headache, dizziness, one hypoglycemic crisis, absence of pubic and axillary hair, testicular atrophy and impotence, enlargement of sella. B.M.R. -45%. Therapy: implantation of testosterone propionate pellets.	1944 1945	3.3 6.0	17 <10
7	N.—Male, 41 yr., fatigue, absence of body hair, anemia, B.M.R. -22%. Enlargement of sella.	1945	3.5 2.6	<10 <10
8	H.—Female, 45 yr., pallor, weakness, anorexia, some loss of weight, amenorrhea since last pregnancy (23 yrs.), hemorrhage following delivery at that time, anemia, absence of axillary and pubic hair, hypoglycemic crises in 1940,—none since then. B.P. 84/60, B.M.R.-30%. In 1944 received 25 mg. testosterone propionate daily. At other times 40 mg. methyl testosterone daily.	1940 1941 1942 1944 1945	1.5 3.9 4.2 13.4 5.4 2.0	<10 <10 <10 <10 <10
9	R.—Female, 47 yr., cachectic, lethargic at time of admission 1944, weakness, absence of axillary and pubic hair, amenorrhea since last pregnancy 17 years ago after severe postpartum hemorrhage. B.M.R. -30%.	1944  1946	1.4 2.3 1.5 1.4	<10 <10 10 <10

## II. Panhypopituitarism (Table 3)

Five cases were studied, three males and two females. All showed many signs of pituitary hypofunction. Of the three males, Case 5 had had a craniopharyngioma which had been removed 22 years prior to the study and cases 6 and 7 showed enlargement of the sella turcica. Cases 8 and 9, both females, gave a history of hemorrhage at the time of labor 23 and 17

years respectively, before study. In all these cases the glycogenic corticoids were markedly low on repeated determinations. In case 8 it is of interest to note that when she had an acute pyelitis with fever the corticoid value was 27 units, a low normal value. This is, however, much lower than the value one would find in a normal individual with an acute infection. As soon as the fever subsided that value fell to less than 10 units. It was raised to 32 units by the administration of pituitary adrenocorticotropin, falling to less than 10 units again on cessation of the injections. This response indicates that the adrenal is still capable of responding to the pituitary after years of hypofunction, and that there probably is in this case a small amount of pituitary tissue left which responds to stress but the quantitative response, as reflected in the urinary corticoids is much less than normal. Since it is quite possible, with partial destruction of the pituitary or adrenal, leading to symptoms of panhypopituitarism or Addison's disease, that the glands are still capable of increasing their secretions under acute stress, the finding in acute infection or after trauma of a value within the limit found in the healthy normal adult, does not necessarily indicate that there is no deficiency.

It is of interest that these individuals did not show marked evidence of electrolyte disturbance in spite of the similarity of their glycogenic corticoid excretion to that found in Addison's disease. This may be further evidence of the possibility of dissociation between two types of adrenal hypofunction. In spite of the low urinary corticoids these individuals ordinarily had normal fasting blood sugars. Case 8 had had repeated hypoglycemic crises in 1940 but had had none since in spite of the occurrence of several infections during which she did not eat for several days and came into the hospital dehydrated. Her blood sugar on two of these occasions on admission was 84 and 78 mg. per cent. As indicated, a small amount of glycogenic corticoids appeared in the urine during the acute infection. Case 6, now followed over eight years, has had only one hypoglycemic crisis, this a severe one in 1944, with semiconsciousness for several days, following an upper respiratory infection. Yet he had been a telephone linesman out in all weathers and eating when he could for years before his one episode of hypoglycemia. This raises the question again at what level of pituitary and adrenal hypofunction does such a disturbance as hypoglycemia arise. Is it below that at which glycogenic corticoids are detectable in the urine by this method? There are probably also other factors entirely outside the endocrine system which may elicit the actual hypoglycemia. One of these could be failure to eat adequately due to the extreme lethargy which often accompanies this disease. However, none of the cases described above, with the exception of Case 9, (patient of Dr.

C. Fullerton), lost much weight or were cachectic in appearance.

In these cases of panhypopituitarism; therefore, the values for glyco-genic corticoids are very low and are comparable to those seen in Addison's disease. Occasionally small amounts were present and in one case an acute infection caused the appearance temporarily of glyco-genic corticoid activity in the urine. The 17-ketosteroids were low but not as low as in the cases of Fraser, et al. (6).

These cases were all treated with thyroid extract and testosterone or methyl testosterone and responded satisfactorily to the therapy.

TABLE 4. ANOREXIA NERVOSA

Case No.	Clinical data	Date	17-Keto-steroids	Corticoids
10	B.—Female, 24 yr., wt. 84½ lbs., loss of weight, asthenia, amenorrhea 2 yr., axillary and pubic hair present, anemia, psychological difficulties. B.P. 100/60. B.M.R. -26%.	1945	mg./24 hr. 7.1 6.4	Gl.U./24 hr. — 36
11	S.—Female, 37 yr., poor appetite, emaciated, lost 44 lbs. from 1928 to 1932, remained between 63–80 lbs. until 1942. Appetite improved and has gained 27 lbs. from 1942–1945. Amenorrhea 13 yr. Axillary and pubic hair present, psychological difficulties. Originally diagnosed as Simmonds' disease, diagnosis changed in 1942 to anorexia nervosa. Lowest B.M.R. in 1932 -27%.	1945	4.0 3.5	17
12	F.—Female, 25 yr., wt. 92 lbs., asthenia, loss of weight, anorexia, headaches, emotional and family difficulties. B.P. 107/94, B.M.R. -21% in 1935. No physical signs of hypothyroidism. In 1938 serum cholesterol = 484 mg.%; in 1944 = 287 mg.%.	1944	7.2	20

### III. Anorexia Nervosa (Table 4)

The differential diagnosis of this condition from that of panhypopituitarism due to organic disease of the pituitary is admittedly difficult. It is generally diagnosed in young individuals with no evidence of pituitary tumor, no history of difficult labor or hemorrhage at labor, no hypoglycemic crises, a retention of pubic and axillary hair, and a history of marked

emotional disturbance with loss of appetite or refusal to eat adequately. Accompanying the loss of weight, there is a low B.M.R. which is followed by amenorrhea and other evidences of pituitary hypofunction. The pituitary hypofunction has been regarded as occurring as a consequence of the malnutrition. Three cases which are regarded as typical as regards history and physical findings were studied. The 17-ketosteroids were low but in general not as low as those in the cases of panhypopituitarism described in the previous group. Glycogenic corticoids were present in detectable amounts in all three cases, ranging from 17 to 36 glycogenic units, values

TABLE 5. HIRsutISM (SIMPLE)

Case No.	Clinical data	Date	17-Keto-steroids	Corticoids
13	W.—Female, 30 yr., obesity, hirsutism face only, irregular menses 6–7 weeks for 1½ yr., previously regular, no clitoral hypertrophy, scalp hair thinning, no true apical baldness.		mg./24 hr.	Gl.U./24 hr.
		1945		
		Mar.	26.2	49
		May	23.1	52
14	M.—Female, 30 yr., excessive hair on face 16 yr., irregular menses, dysmenorrhea, gained 150 lbs. between ages of 12 and 15 yr. due to increased appetite. In 1939 placed on 1200 cal. diet, lost 150 lbs. over 10 mo. Cholelithiasis in 1940, no clitoral hypertrophy, pregnandiol present from 16th to 27th day of a 28 day cycle. Total amt. 26 mg.	1945	29.7	54
15	L.—Female, 16 yr., facial, chest, and abdominal hirsutism 2 yr., hair growth on arms and legs increased, scalp hair falling out, acne, no clitoral hypertrophy, irregular menses 6–8 weeks, pregnandiol present in luteal phase.	1945	27.8 37.0	65
16	M.—Female, 54 yr., excessive hair on face, markedly obese, amenorrhea 5 mos., hot flushes. Hypertensive cardiovascular disease. B.P. 180/130. Generalized arteriosclerosis. Osteoarthritis of phalanges, fibromyoma ovary, uterine fibroids, at operation adrenals explored, right adrenal somewhat enlarged.	1945	27.0	38

which are normal or slightly below normal. Both in the group of panhypopituitarism with organic lesions and this group, there is an indication of a quantitative difference in degree of reduction of adrenal function, in relation to varying degrees of reduction of pituitary function due either to more or less destruction by an organic lesion or to varying degrees of functional impairment as a response to malnutrition or some other influence outside the pituitary itself.

#### IV. Hirsutism—Simple (Table 5)

Four cases of simple hirsutism are presented. None of these patients showed any signs of Cushing's syndrome or other signs of virilism. In two of them, although the menses were irregular, a luteal phase was present as evidenced by the presence of pregnandiol at the expected time in the cycle. The 17-ketosteroids were definitely raised in all four cases, while the glycogenic corticoids were normal or only slightly above normal. Comparing the 17-ketosteroid and glycogenic corticoid values in these cases with those of Cushing's syndrome, it is obvious that these two values can vary independently. Thus the 17-ketosteroids may give evidence of an increased adrenal function while the corticoids remain normal and the reverse condition may also occur.

#### V. Cushing's Syndrome (Table 6)

As has been mentioned, Anderson, Haymaker and Joseph first showed the presence of life-maintaining corticoids in the blood and urine of a case

TABLE 6. CUSHING'S SYNDROME

Case No.	Clinical data	Date	17-Ketosteroids	Corticoids
17	A.—Female, 33 yr., weakness, hirsutism, amenorrhea since last pregnancy 13 mo., plethoric appearance, tendency to bruise, ecchymosis of both lower legs, osteoporosis of vertebrae, large but normal sella. B.P. 150/100. Glucose tolerance test—Fasting B.S. 84; 30 min. 172; 60 min. 152; 120 min. 148; 180 min. 119 mg. %. Insulin resistant. Operation—left adrenal containing benign adenoma size of walnut removed. Autopsy showed atrophy of right adrenal with small cortical adenoma.	1944	mg./24 hr. 10.0 8.1	Gl.U./24 hr. 700 500

TABLE 6. (Continued)

Case No.	Clinical data	Date	17-Keto-steroids mg./24 hr.	Corticoids Gl.U./24 hr.
18	C.—Female, 42 yr., headache, increasing facial hirsutism and obesity 12 yr., weakness, drowsiness, irregular menses, plethoric appearance, osteoporosis, hypertrichosis of face and trunk. B.P. 230/135. Glucose tolerance test—fasting B.S. 126; 30 min. 199; 60 min. 262; 120 min. 175; 180 min. 130 mg.%. X-ray showed no abnormality of sella turcica.	1945	20.4 22.2 23.6	340 212 316
19	C.—Male, 11 yr., Symptoms had developed within a year, plethoric appearance, obesity with typical moon face, large hump in the upper dorsal region, moderate osteoporosis especially in lumbar spine, definite striae of the skin, normal genital development with some scant pubic and axillary hair. The sella was normal in size. Blood sugar 116 mg.% 1½ hr.p.c. Exploration of the adrenals showed no distinct abnormality.	1946	11.5	386 218
20	S.—Female, 39 yr., increasing facial hirsutism and acne with plethoric appearance 6 mo. Raised B.P. and protuberance of abdomen noted 1 year previously. Glucose tolerance tests of diabetic type. Relatively little osteoporosis. Sella turcica normal. Operation 2 weeks prior to assays—right adrenal removed—2½ times normal size—contained adenoma which had obliterated medullary space.	1946	26.1	137
21	F.—Male 27 yr., obesity and plethoric appearance 15 yr., hemorrhage in retinae. B.P. 210/140, mild osteoporosis of spine and pelvis. Glucose tolerance was within normal range. X-ray therapy in May 1943 with no improvement. Left adrenal and part of right adrenal removed in October 1943. No abnormality found. Still shows clinically the same evidences of Cushing's syndrome as before operation.	1946	11.0	42

of Cushing's syndrome. In 1939 Weil and Browne found by the cold exposure test an amount of urinary corticoid above normal in a male with typical Cushing's syndrome. In this same patient in whom the symptoms and signs of the disease disappeared, there was found a normal value by the cold exposure test in 1941 and again in 1945 by the glycogen assay. Eggleston and Dobriner (4) reported urinary corticoids in three cases of Cushing's syndrome; two showed markedly raised values, the third, normal values.

Five cases are presented in this study. In case 17 (Dr. Fuller Albright's patient), the glycogenic corticoids were very high, 500-700 units per twenty-four hours. The 17-ketosteroids were not raised. Assays on this patient's urine were also performed in Dr. Dobriner's and Dr. Albright's laboratory, both groups found values markedly above normal, Case 18 (a patient of Dr. E. H. Mason), was a typical case of several years duration. Operation some years previously at the Mayo Clinic had revealed no gross abnormality of the adrenals. The 17-ketosteroid values were slightly above normal, the glycogenic corticoids were markedly raised, varying from 160 to 340 units per 24 hours. Case 19 (Dr. E. Perry McCullagh's patient), a boy of 11 years, in whom the symptoms were of recent development, had 17-ketosteroids within normal range for his age. The corticoids were greatly increased, 218-386 glycogenic units. Case 20 (Dr. E. Perry McCullagh's patient), a woman of 39 years with typical appearance of Cushing's syndrome—most of her symptoms and signs had developed within the last year—showed an increased excretion of corticoids and 17-ketosteroids. In case 21 evidences of Cushing's syndrome had been present for 12 years. His left adrenal and part of the right adrenal had been removed in 1943, and although he was in much better condition in 1946 than before the operation, he still showed clinically the same evidences of Cushing's syndrome as before. The 17-ketosteroids and corticoids were normal.

From the present findings and those of others, it is apparent that cases of active Cushing's syndrome show high values for glycogenic corticoids. In certain patients in whom the process has been arrested or has become inactive, normal values are found. The values may be increased either with or without the presence of an adrenal tumor. The rise in glycogenic corticoids is not necessarily accompanied by a rise in 17-ketosteroids.

It is of interest that in case 17 the glucose tolerance curve was only slightly impaired though she was insulin resistant, whereas the glucose tolerance curves in cases 18 and 20 were definitely impaired. The glycogenic corticoids were higher in the first case. It is also true that in other conditions in which the urinary glycogenic corticoids may be much in-

creased, for example in pregnancy and after trauma and surgical operations, a raised urinary corticoid value and a normal glucose tolerance curve may coexist in the same patient at the same time. This is another illustration of the fact mentioned in the introduction that a metabolic test such as glucose tolerance is influenced by many factors and cannot be used as an index of any single glandular function.

While we have not had the opportunity of studying any cases of adrenal cortical tumor, those that have been reported in the literature usually show markedly increased 17-ketosteroids and Eggleston and Dobriner (4) have reported that the glycogenic corticoids were also increased in two cases of adrenal cortical carcinoma.

## VI. Acromegaly (Table 7)

Four cases of acromegaly are presented. Cases 22 and 24 were of long standing and both showed normal values for glycogenic corticoids. The 17-ketosteroids were normal in case 22 and only slightly increased in case 24. The latter case had shown a marked impairment of sugar tolerance existing for several years. In 1945 he developed symptoms of polyuria and polydipsia. The symptoms and the blood glucose values were controlled by diet alone, although lately he has required small doses of insulin. This is not characteristic of the type of carbohydrate disturbance frequently seen in acromegaly which is usually insulin resistant. Case 23

TABLE 7. ACROMEGALY

Case No.	Clinical data	Date	17-Keto-steroids	Corticoids
22	S.—Female, 54 yr., gradual enlargement of hands, feet and face over a period of 15 years, headaches, insomnia, fatigue increasing 5 years. X-ray shows enlarged pituitary fossa with calcification of pituitary gland. Glucose tolerance test impaired. B.P. 210/90. X-Ray therapy in 1944.	1945	mg./24 hr. 12.7	Gl.U./24 hr. 26
23	D.—Female, 32 yr., severe headaches and amenorrhea 3 years, enlargement of hands, feet and face 1½ years. X-ray showed enlarged sella pituitary tumor. Radiation of pituitary in 1944. Chromophobe adenoma of pituitary removed in 1945.	1945	11.8	62



TABLE 7. (Continued)

Case No.	Clinical data	Date	17-Keto-steroids	Corticoids
24	J.—Male, 39 yr., marked prognathism, extremely large hands, feet and tongue. Received deep X-ray in 1932 for two years which arrested progression of symptoms. Diabetes mellitus controlled by diet. Glucose tolerance curve—Fasting B.S. 172; $\frac{1}{2}$ hr. 314; 1 hr. 432; 2 hr. 312; 3 hr. 196 mg.%. Not insulin resistant.	1945	mg./24 hr. 26.3	Gl.U./24 hr. 53
		1946	24.0	40
25	G.—Male, 28 yr., fatigue and somnolence 2 years. Pain in neck, head and face 2 years. Decreased sexual function 8 months. Prognathism, terminal tufting of phalanges. X-ray showed marked enlargement of sella. Glucose tolerance test normal. B.M.R. -11%.	1946	37.6 28.6	134

(a patient of Dr. W. Penfield), with acromegaly of relatively short duration, also showed normal values for glycogenic corticoids, and had received irradiation to the pituitary region in 1944. Case 25 (a patient of Dr. W. Penfield), an untreated case of recent origin, showed glycogenic corticoids of the order of 134 units, which is definitely raised. The glucose tolerance test was normal in this case. The 17-ketosteroids were also increased.

In this group of cases there is no correlation between the level of glycogenic corticoids and the disturbance of carbohydrate metabolism. However, signs and symptoms of the disease can exist without evidence of increased adrenal cortical function as measured by the glycogenic corticoids. The cases are too few to draw definite conclusions.

## VII. Diabetes (Table 8)

Several cases of diabetes have been studied and all have shown normal values. The two cases presented are of interest in that their diabetes was difficult to control. Case 26 (patient of Dr. E. H. Mason) readily developed acidosis. The urine was collected following a period of acidosis when large amounts of insulin were being administered, and hypoglycemia was present part of the time. The corticoids were normal. Case 27 (patient of Dr. W. de M. Scriver) showed signs of increasing insulin resistance and the

TABLE 8. DIABETES

Case No.	Clinical data	Date	17-Ketosteroids	Corticoids
26	B.—Female, 27 yr., diabetes since 1929. Develops acidosis readily. Admitted to hospital in acidosis and ketosis. B.S. 335 mg.%. CO <sub>2</sub> 18.8, vols.%. Received a total of 35 units P.Z. insulin, and 128 units C.Z. insulin. Urine collected for the next 48-hours for corticoids assay. Jan. 25—received a total of 56 units C.Z. insulin. B.S. a.c. 37 mg.%; p.c. (4 hrs.) 46 mg.‰—no glucosuria but acetofuria present—insulin reaction in p.m. Jan. 26—insulin reaction in afternoon, no glycosuria.	1945	mg./24 hr. 10.0	Gl.U./24 hr. 45
27	L.—Male, 59 yr., diabetes since 1934, insulin requirements 28 units daily. Has never been in diabetic coma. 3 weeks previously became confused and ran car into ditch. 3 days after accident patient vomited several hours, admitted to hospital in acidosis. B.S. over "1000." Since that time diabetes has proved impossible to control and patient has showed signs of increasing insulin resistance. Corticoid assay done 5 weeks after accident. Receiving 8 units P.Z. insulin and 32 units C.Z. insulin. B.S. a.c. 307; p.c. 358 mg.%. No glycosuria.	1946	10.8	54

diabetes was difficult to control. At the time of assay the blood sugar was above 300 mg. per cent. The 17-ketosteroids and corticoids were found to be normal.

#### VIII. Miscellaneous Cases (Table 9)

Case 28 was one of premature sexual maturity. There was no evidence of gross enlargement of the adrenal in the retrograde pyelogram but no retroperitoneal air injection or exploration of the adrenals was done. By pneumoencephalogram no evidence of intracranial lesion could be detected. The 17-ketosteroids were raised for a child of this age but the urinary corticoids were within normal limits. Case 29 was one of marked undergrowth and development in a child of 2½ years. There was no x-ray evidence of gross lesion of the pituitary. The diagnosis of pituitary dwarf-

ism at this age is difficult but, as no obvious cause for her undergrowth was found, this diagnosis was tentatively made. The 17-ketosteroids and corticoids were within normal limits. The history of rapid growth in case 30 plus the evidence of the enlargement of the sella would suggest the presence of a pituitary tumor with active secretion of the acromegalic gigantism type. A hypofunctional state is indicated, however, by the presence of a slow pulse, a basal metabolic rate of  $-32$  per cent, low blood corticoids, disappearance of body hair, and the delayed epiphyseal closure. This evidence suggests that the tumor is a non-functioning one and has by its enlargement destroyed the tissue of the pituitary so that the individual is at present suffering from panhypopituitarism. It is possible that the output of growth hormone was temporarily increased as the tumor began to grow. Case 31 has been diagnosed as Addison's disease because of the weakness, typical pigmentation, including mucous membrane pigmentation, and lowered blood pressure. The condition had not responded to DCA therapy over some time. It was obvious that he had an enlarged firm liver and there were other evidences of cirrhosis. The chest x-ray suggested the presence of neoplastic metastases but a primary lesion was not detected in the gastro-intestinal or urinary tract. He was suffering from marked malnutrition and had had anorexia, nausea and vomiting for some time. The impaired glucose tolerance curve was against a diagnosis of Addison's disease. Hemochromatosis was suggested; the pigmentation in the skin was melanin but cases of hemochromatosis are reported in which this has been found. The 17-ketosteroids were low but the corticoids were

TABLE 9. MISCELLANEOUS CASES

Case No.	Clinical data	Date	17-Ketosteroids mg./24 hr.	Corticoids Gl.U./24 hr.
28	PRECOCIOUS SEXUAL DEVELOPMENT. A.—Male, $2\frac{1}{2}$ yr., height $43\frac{1}{4}$ in., weight 47 lbs. Rapid physical and precocious sexual development began at age 1, testes and secondary sex organs adult size, axillary and pubic hair present, acne, deep voice.	1945		
		May	12.5	55
		Nov.	10.7	41
		1946		
		July	5.6	42
29	PITUITARY DWARFISM. Female, $2\frac{1}{2}$ yr., height $29\frac{1}{4}$ ins., poorly developed, under nourished and under weight. "Growth arrest" lines in long bones.	1946	3.2	48

TABLE 9. (Continued)

Case No.	Clinical data	Date	17-Keto-steroids	Corticoids
30	<b>CHROMOPHOBE ADENOMA PITUITARY.</b> J.—Male, 24 yr., has grown $3\frac{1}{4}$ in. and gained 100 lbs. in last 5 yr. Headaches 3 yr. Blurring of vision $1\frac{1}{2}$ yr. Dizziness. Loss of body and axillary hair. Normal pubic hair. Enlarged sella and probable chromophobe adenoma. B.M.R. -33%. Glucose tolerance—Fasting B.S. 57; $\frac{1}{2}$ hr. 117; 1 hr. 75; $1\frac{1}{2}$ hr. 52; 2 hr. 68; $2\frac{1}{2}$ hr. 74 mg.%.	1946	mg./24 hr. 9.5	GLU./24 hr. 15
31	<b>HEPATIC CIRRHOSIS.</b> S.—Male, 62 yr., weakness $1\frac{1}{2}$ yr. Loss of weight, drowsiness, vomiting, melanin pigmentation of skin and oral mucosa, enlarged firm liver, impaired liver function, raised plasma globulin, serum Na 141 m.eq./l. B.P. 95/60, impaired glucose tolerance, no response to D.C.A. therapy.	1945 June Sept. 8 Sept. 18 Oct. 12	7.0 7.5 8.4	44 47 117 49
32	<b>HYPERTHYROIDISM.</b> G.—Female, 49 yr. Thyroidectomy in 1936 for nodular toxic goiter. 3 years later regrowth of thyroid. 1946 B.M.R. +48%, amenorrhea since last pregnancy 1934. Weakness, nervousness, loss of weight. No axillary hair, slight pubic hair. Brownish pigmentation of skin,—none on mucous membrane. Cholesterol 106 mg.%. Serum Na. 141 m.eq./l.	1946	6.7	55
33	<b>HYPOGONADISM.</b> I.—Male, 40 yr. Weakness 8 yrs. Hot flushes 4 years. Occasional nausea and vomiting. Absence of facial, body and axillary hair. Scant pubic hair. Skin pale, does not tan. Testes small and soft. No libido.	1946	6.7 4.1	61
34	<b>CHRONIC ASTHENIA.</b> C.—Male, 44 yr. Fatigue, weakness, nervousness 15 yrs. Unable to work, underweight. B.M.R. -15- -25%. Sensitive to cold. Genitalia underdeveloped. Testes small. Hair distribution is normal. Recurrent depression. B.P. 125/70.	1946	18.3 16.2	64

normal and later rose as high as 117 units for a period. This case illustrates the possible usefulness of this estimation in excluding the presence of an adrenal deficiency in an obscure case with some of the features of Addison's disease. In case 32 the question of panhypopituitarism or Addison's disease was raised. The absence of axillary hair had apparently been present prior to the hemorrhage at labor but the history was not definite on this point. It seemed unusual for panhypopituitarism to coexist with hyperthyroidism and regrowth of a toxic goitre. The normal urinary corticoids were against a diagnosis of Addison's disease or marked panhypopituitarism. Case 33, aged 40 years (a patient of Dr. E. S. Mills), was a typical case of hypogonadism with no secondary development and small testes. He was normally grown and there was no x-ray evidence of tumor of the pituitary. The 17-ketosteroids were low but the corticoids were normal. This suggested that the pituitary was normal in so far as the adrenotropic function was concerned. Case 34 was one of marked asthenia. This asthenia had been present for years. The patient was neurotic and had a prolonged history of medical examinations with various diagnoses, among them Simmonds' disease and atypical myasthenia gravis. He was poorly developed and poorly nourished. He had been regarded as a hypogonad, his penis and testes were somewhat small but body hair growth was normal and he shaved every day. He showed no abnormal skin pigmentation and his blood pressure was 125/70. The 17-ketosteroids of 18.0 mg. and corticoids of 64 g.u. were within normal limits. These findings were against gross abnormality of the adrenal or pituitary and were thus of assistance in an obscure chronic case of asthenia with marked psychiatric features.

#### SUMMARY

In general then it may be said that the determination of urinary glyco-genic corticoids in addition to the 17-ketosteroids is of assistance in elucidating various types of cases whose signs and symptoms suggest abnormality of adrenal function. The two determinations frequently do not parallel each other and do not give the same type of information regarding adrenal function. Both are indirect estimates of this function and are subject to various sources of error and to being influenced by factors other than adrenal cortical secretion. The biological determination of glyco-genic corticoids has the advantage that it measures one type of adrenal cortical substances, those affecting carbohydrate and protein metabolism, and so far as is known, substances having this type of activity are derived only from the adrenal cortex.

It has been pointed out that there are three main types of adrenal cortical function and that it is possible that they may vary independently of one another so that the clinical signs and symptoms produced and the

metabolic findings may vary, leading to a variety of syndromes all associated with hyper or hypo function of the adrenal cortex. Physiological states, such as pregnancy and muscular exercise, are accompanied by an increased adrenal function as measured by this test, and following trauma, infection, and surgical operations, there is a rise in excretion of these substances.

It is obvious that clinical and metabolic changes ordinarily associated with variations in adrenal function may be affected by a variety of other factors. In so far as the measurement of urinary glyco-genic corticoids does reflect a type of adrenal function, it may give indications of alteration in adrenal function in both physiological and pathological conditions in which other methods, such as the estimation of 17-ketosteroids or the ratio of  $\alpha$  to  $\beta$  ketosteroids, do not. Thus the role of the adrenal cortex in these conditions may be further elucidated, not only in those classical clinical syndromes with which it has been associated but also in the regulation of body functions in response to exposure, to diseases other than those of the endocrine system, and to stress, which has been emphasized by Selye (9) in his work on the Adaptation syndrome.

### CONCLUSIONS

The use in various types of endocrine and other clinical disorders, of the bioassay of adrenal cortical substances in urine, having the property of depositing glycogen in the liver of the adrenalectomized mouse, has been described. This has been compared with the estimation of the total neutral 17-ketosteroids.

The glyco-genic corticoids and the 17-ketosteroids were in general low or not detectable in cases of Addison's disease and in panhypopituitarism with organic lesion of the pituitary.

In three cases diagnosed as anorexia nervosa the 17-ketosteroids were low but not as low as in panhypopituitarism with organic lesion. The glyco-genic corticoids were normal or slightly below normal.

In four cases of active Cushing's syndrome the glyco-genic corticoids were greatly increased with the 17-ketosteroids normal or slightly increased. In arrested cases the glyco-genic corticoids may be within normal limits.

In four cases of simple hirsutism the 17-ketosteroids were increased and the glyco-genic corticoids within normal limits.

In acromegaly in three long established cases the 17-ketosteroids and glyco-genic corticoids were normal. In one recent untreated case the 17-ketosteroids were at the upper limit of normal and the urinary corticoids twice normal.

The ketosteroids and glyco-genic corticoids frequently do not parallel

each other. The one may be high and the other normal or low or the reverse situation may occur.

A group of miscellaneous cases is presented indicating the usefulness of the glycogenic corticoid determination in obscure cases suspected of having adrenal dysfunction. Cases having some symptoms and signs of adrenal deficiency with normal or raised glycogenic corticoids are presented.

A general review of various methods of measuring adrenal cortical function in clinical conditions is presented and the sources of error of each are discussed.

It is suggested that the three main types of function of the adrenal: (1) electrolyte metabolism, (2) protein and carbohydrate metabolism and (3) androgenic production, may vary independently of one another both qualitatively and quantitatively, thus leading to a variety of metabolic conditions and clinical syndromes. The use of the determination of glycogenic corticoids offers a further method of estimating one type of adrenal cortical function in health and disease.

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# HYPEROPHTHALMOPATHIC SYNDROME IN THYROID DISEASE

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**I**N RECENT years attention has been redirected to cases of thyrotoxicosis with predominance of ophthalmic manifestations. The latter include proptosis, paresis of external ocular muscles, swelling of the lids, edema of the conjunctivae and retrobulbar pain. A variety of designations has been applied to this syndrome, such as "malignant exophthalmos" and "exophthalmic ophthalmoplegia." These terms are purely descriptive and stress one or another feature of the syndrome, not all of which are present in every case. Recently two other terms have been suggested. Mulvany (12) speaks of "thyrotropic exophthalmos" a term that is objectionable on linguistic grounds since the author evidently does not wish to imply that the exophthalmos is thyrotropic but that it is caused by thyrotropic hormone. It would then be thyrotropogenic rather than thyrotropic. Means (9) speaks of "hyperophthalmopathic Graves' disease." Mulvany's term implies a very definite opinion as to the genesis of the syndrome whereas Means is deliberately noncommittal on this point but implies that the cases in question cannot be absolutely separated from "classical" Graves' disease.

The following cases illustrate certain practical therapeutic problems. Some observations made in these patients appear of basic interest and may shed some light on the pathophysiology of the exophthalmos.

**Case 1.** J. G., a white man aged 43, was admitted to Jefferson Hospital Jan. 21, 1944. In the summer of 1943 he developed thyrotoxic manifestations (nervousness, excessive sweating, frequency of bowel movements, insomnia, palpitations). He claims that his eyes protruded rather suddenly following an alcoholic debauch. The condition became increasingly worse and he was admitted to another hospital October 1, 1943. The BMR was at that time +85, the basal pulse rate 126. After routine preparation with potassium iodide, subtotal thyroidectomy was performed at a time when his BMR was +46 (pulse 96). He believes that his eyes improved somewhat following the operation. However, soon after December 1943 the ocular condition deteriorated considerably.

On admission, the lids of both eyes were swollen and ectropionized, exposing the entire conjunctivae. The eyeballs were bulging, the conjunctivae edematous. There were ulcerations on both corneae and there was considerable photophobia. He complained of severe "headaches" localized "behind the eyes." The eyeballs were very tender to pressure and almost entirely immobile, upward movement being impossible, downward and sideward movements considerably limited. Pressure on the eyeballs within the limits possible because of pain, showed that they could not be pushed into the orbit (Fig. 1).

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103

## HYPEROPHTHALMOPATHIC SYNDROME

He was conspicuously slow in reacting to questions, his voice was raucous, his skin and hair dry, face and extremities showed a rather hard non-pitting swelling. His blood pressure was 120/80. The BMR was minus 21; serum cholesterol 161 mg. per cent; serum bilirubin 0.2 mg. per cent; phenolsulphonephthalein test: all dye removed. RBC 3.9 Mill. Hb. 84 per cent, WBC 6,900 with normal differential count. X-ray of the skull was negative. Urinary hormone excretion: gonadotropins non-demonstrable, estrogens less than 6 IU. per 24 hours, 17 ketosteroids 4.5 mg. equivalent of androsterone per 24 hours.

The diagnosis of severe ophthalmopathy ("inalignant exophthalmos") was clear



FIG. 1. Case 1. J.G. Before treatment (January 1944).

enough. It also seemed clear that conservative measures would not become effective quickly enough to save the endangered eyes. As a first emergency measure a canthotomy and scarification of the cornea was performed and celluloid cups were placed over both eyes (by Doctor Charles E. G. Shannon, Department of Ophthalmology). Subsequently a decompressing operation (Kroenlein-Naffziger) was performed by Doctor R. Jaeger (Department of Neurosurgery), on the right side on February 4, 1944, and on the left side on March 9, 1944.

Thyroid medication was started on the day following admission. He received 0.06 gram (1 grain) daily for one week, then 0.09 gram ( $1\frac{1}{2}$  grains) for one week, 0.12 gram (2 grains) for two weeks and 0.18 gram (3 grains) from then on. X-ray irradiation of the pituitary gland was started two days after admission, and within 10 days 900 r were applied to each field.

The clinical effect of the treatment was gratifying. The patient left the hospital on March 21 greatly improved. Treatment was continued in the Endocrine Clinic (out-patient department) with thyroid U.S.P. 0.18 gram (3 grains) daily. Figure 2 shows the result of treatment.

While the ultimate result of therapy was satisfactory the unusually slow response to thyroid medication was remarkable. On March 17 after 55 days of treatment with thyroid the BMR had hardly risen (minus 17 on March 17). By June 5, after 164 days of treatment which included one intravenous injection of thyroxin in addition to the oral medication the BMR had risen to minus 2. On June 15, fifteen mg. of crystalline thyroxin were injected intravenously. As can be seen from Figure 3 this large dose had no metabolic effect. Neither were there any other effects such as tachycardia or vasomotor phenomena.



FIG. 2. Case 1. J.G., After fifteen months of treatment (April 1945).

*Summary of Case 1.* A 43 year old patient developed a most severe hyperophthalmopathic condition following subtotal thyroidectomy for thyrotoxicosis. From this truly nalignant ophthalmopathy he made satisfactory recovery after a decompressing operation, administration of thyroid extract, and irradiation of the pituitary gland. The patient showed a very poor metabolic response to thyroid medication, both oral and intravenous.

*Case 2.* C. F. a white woman aged 42, was admitted to the Endocrine Clinic on August 23, 1943 with the following history. She had been operated upon in another hospital for thyrotoxicosis two years previously. According to the report subsequently obtained from this hospital she had presented a typical picture of Graves' disease with a BMR of plus 35 and moderate exophthalmos.

The operation evidently was successful in alleviating the thyrotoxic manifestations. Her nervousness subsided, she gained weight and felt well. The condition of her eyes, however, deteriorated. The protrusion increased considerably and her lids became swollen.

On physical examination the ocular findings were very conspicuous. There was con-

siderable exophthalmos (exophthalmometer readings 23 mm. right eye, 20 mm. left eye); the lids were swollen; there was paresis of upward gaze. The bulbi were tender to palpation and attempts at ocular movements in all direction were painful. There was considerable photophobia.

She was a moderately obese woman. There were no other findings of note. Her menses were regular.

The BMR was plus 1 (basal pulse rate 70); serum cholesterol 259 mg. per cent; 17-

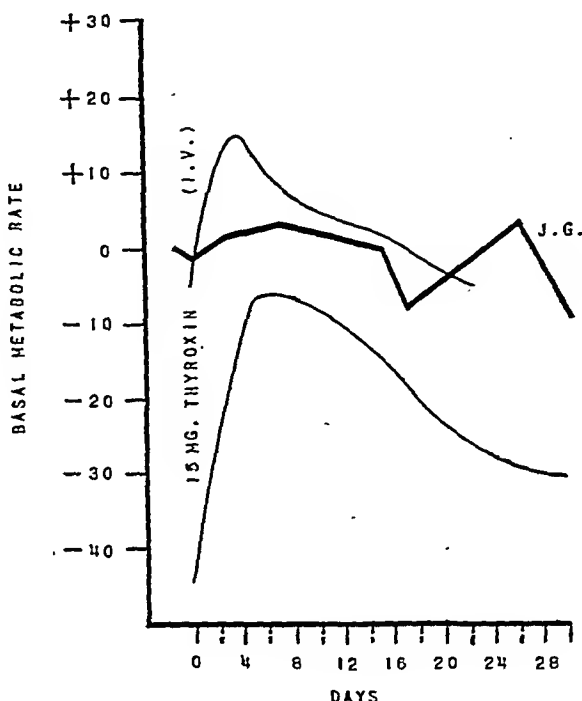


FIG. 3. Response of J.G. (Case 1) to intravenous injection of 15 mg. of crystalline thyroxin. For comparison the response of a myxedematous patient at two different levels of BMR to 10 mg. of thyroxin I.V. is given. These curves are redrawn from the paper of W. O. Thompson et al. (22).

ketosteroid excretion 7.6 mg. equivalent of androsterone per 24 hours; gonadotropin excretion varied from 0 to 42 IU per 24 hours.

Treatment was started on September 27, 1943, with thyroid U.S.P. 0.09 gram (1½ grains) daily, and after one month this was increased to 0.12 gram (2 grains) daily. After five months of treatment, by March 1944, photophobia had completely subsided, and the swelling of the lids while still present was considerably lessened. The eyeballs had visibly receded, and the exophthalmometer readings now were 17 mm. on the right eye, and 15 mm. on the left eye. The BMR, however, was unchanged, plus 1. Serum cholesterol was 171 mg. per cent. The daily dose of thyroid was now increased to 0.18 gram (3 grains), and soon thereafter to 0.24 gram (4 grains). After one year of treatment her condition was greatly improved but now appeared stationary. A course of x-ray

irradiation was given to the pituitary gland (900 r) with the hope of inducing further regression of the exophthalmos. This did not appear to have a striking result.

Treatment with oral doses of thyroid U.S.P. 0.24 gram (4 grains) daily was continued, and two more courses of irradiation to the pituitary were given. Further improvement was obtained but full normalization was not achieved.

*Summary of Case 2.* A woman aged 42 was operated upon elsewhere for a moderately toxic goiter. In the two years following subtotal thyroidectomy the exophthalmos increased considerably and other manifestations of ophthalmopathy developed. She was treated with desiccated thyroid and x-ray irradiation of the pituitary gland. Her condition was greatly improved but did not return to normal.

*Case 3.* J. C., a white woman aged 33, was referred by her family physician to surgical service (A.) because of exophthalmos. Eight months prior to this admission she had become irritable following forceps-delivery of her second child. The pregnancy and delivery had been uneventful. Exophthalmos was noticed four months after the approximate onset of nervousness. It increased rather rapidly and was a prominent feature when she was first seen in the hospital. Complaints referable to thyrotoxicosis were mild; there was some increase in appetite, hardly any weight loss, no palpitations, and no increased perspiration. Menarche had occurred at the age of 14, her periods were regular at 27-days interval, of 4-5 days duration.

The history was otherwise non-contributory except that a cousin allegedly had thyrotoxicosis at the age of 14.

Physical examination showed the thyroid barely palpable and smooth. There was a moderate degree of hypertrichosis, hair growth being present on chin and lip (this hirsutism had been present in the same degree since puberty). There was no tremor and her pulse rate was 80 to 90, the blood pressure was 120/70. The severe exophthalmos was the most striking feature. (Exophthalmometer reading 18 mm. on both eyes.) Fundi were normal. There was paresis of upward gaze and paresis of convergence. Any attempt to move the eyeballs was associated with considerable pain, and patient turned her head rather than her eyes when attempting to look sideways or upward. On pressure the bulbi were tender, and could not readily be pushed back into the sockets. There was, however, no edema of the conjunctivae. Considerable photophobia was present. She weighed 119 pounds.

Her BMR was plus 14 (basal pulse rate 76); her serum cholesterol 234 mg. per cent; the urine analysis negative for sugar and albumen. The blood count was normal. X-ray of the skull revealed no abnormality. 17-ketosteroid excretion was 5.7 mg. equivalent of androsterone per 24 hours. Estrogen excretion was 16 MU per twenty-four hours, gonadotropins non-demonstrable.

In view of the absent, or at the very most, mild, thyrotoxic manifestations (some nervousness, slightly increased appetite) as contrasted to the marked ocular manifestations, operation was advised against and patient was transferred to the Endocrine Clinic. On August 11, 1943, treatment was started with diethylstilbestrol, 2 mg. daily. Two weeks later she stated that she felt much relaxed and generally better but there was no distinct improvement of the eyes. The daily dose was increased to 3 mg., and on September 8 to 4 mg. daily. On September 22, five and one-half weeks after treatment with diethylstilbestrol had been started the exophthalmos was clearly receding and exophthalmometric measurements now were 12 mm. on the left and 13 mm. on the right eye. The rather large doses of diethylstilbestrol were tolerated without any discomfort or toxic manifestations. Stilbestrol apparently failed to suppress pituitary gonadotropin production. An assay performed at this time showed an excretion of 16 IU per twenty-

four hours. On October 15, 1943, the daily dose was increased to 7 mg. About this time she noticed further and considerable improvement now being able to "turn her eyes rather than to have to turn her head." Her eyes felt "relaxed." She had gained five pounds (125½) and felt completely well. A BMR was determined at this time as a routine checkup, and a reading of plus 52 was recorded, with a basal pulse rate of 80. The serum cholesterol was 146 mg. per cent. The BMR determination was repeated two weeks later with identical result (plus 53, pulse 96). Because the patient felt entirely well and had no thyrotoxic manifestations, and because the ocular signs had been greatly improved, the medication was continued. In February, 1944, her BMR was plus 56 (pulse 84); her serum cholesterol 140 mg. per cent. Glucose tolerance test showed the following values: fasting blood sugar 109 mg. per cent, ½ hour after oral ingestion of 100 grams, glucose 167 mg. per cent, 1 hour 156 mg. per cent, 2 hours 122 mg. per cent. The patient had now had high basal metabolic rates and low serum cholesterol values for four months but again the absence of thyrotoxic manifestations both subjective and objective was noted. The weight had further increased to 129 pounds. The estrogen excretion was at this time found to be high (more than 100 MU per 24 hours). The serum estrogen was 60 MU/100 cc. Urinary gonadotropins were not demonstrable.

It was now decided to withdraw stilbestrol medication in order to evaluate a possible influence of the estrogenic compound in producing this unusual picture. In order to avoid massive withdrawal bleeding the withdrawal of the drug was carried out gradually beginning on February 25, 1944, and finally discontinued entirely on March 17, 1944. On March 31, 1944, the BMR was plus 53 (pulse 90). Thyrotoxic manifestations remained absent. On May 12 her BMR was plus 22 (pulse 80), her serum cholesterol 146 mg. per cent. In the next several months the BMR varied between plus 20 and plus 30. Her weight increased to 136 pounds. She felt well and the improvement of the ocular manifestations was maintained.

In April, 1945, as a therapeutic test, she received Lugol's solution, 15 drops daily for fourteen days. Her BMR fell to plus 7. Clinically there was no change, and when questioned she stated that she had felt so perfectly well prior to the iodine medication that she could not report any "improvement." In June 1945, six weeks after discontinuing iodine medication, the BMR had risen to plus 31 (pulse 84). In spite of the absence of clinical manifestations of thyrotoxicosis it was deemed inadvisable to wait for such to appear. Treatment with thiouracil was started on June 18, 1945. At first the drug was well tolerated but on August 8 she was admitted to this hospital with a very severe agranulocytosis. The drug was immediately withdrawn and treatment with penicillin and large doses of Vitamin B (Brewers Yeast) given. She recovered completely.

Since that time her condition has remained stationary. She has had two more courses of x-ray treatment to her pituitary gland (600-900 r. each). Her weight is constant, she feels well but still has a moderately elevated BMR (plus 20).

*Summary of Case 3.* A woman aged 33 years was observed with very mild (border line) thyrotoxic manifestations and considerable ophthalmopathy. Treatment with large doses of diethylstilbestrol resulted in marked improvement of the ophthalmopathy. During the period of treatment with the estrogenic compound, at a time when her eyes were considerably improved, her BMR rose and the serum cholesterol decreased but there were no clinical manifestations of thyrotoxicosis. Diethylstilbestrol was withdrawn. The BMR decreased without, however, reaching normal levels, and the serum cholesterol remained low. A therapeutic-diagnostic test with iodine medication resulted in prompt fall of the BMR to normal values, with a subsequent rise after discontinuing iodine medication.

**Case 4.** E. C., a white man aged 49, in October 1943 first noticed double vision and difficulty in gazing upward. The latter was limited to the left eye. At about the same time he became very nervous, perspired excessively and developed tremor. His appetite was poor, and he lost 20 pounds in a few months. He was seen by an internist whose studies revealed the following: BMR plus 30 (basal pulse rate 96); serum cholesterol 104 mg. per cent; X-ray of the skull negative; pulse rate 100 to 120; Wassermann and Kahn reaction in the serum negative. Spinal fluid examination was normal. There was marked tremor of the hands. Both eyes showed limitation of outward rotation, slight limitation of inward movement and marked impairment of upward gaze bilaterally, but more marked on the left. Myasthenia gravis was considered but there was no response to prostigmin. Thiouracil treatment was started but had to be discontinued after 10 days because of drug fever and rash.

During the following year the patient was under the care of his family physician, and received symptomatic treatment only (sedatives). He was then referred to the surgical service of Doctor T. A. Shallow for thyroidectomy. Upon admission his condition was the same as described above; his ophthalmopathy had neither improved nor deteriorated, he still had tachycardia (120), his BMR was now plus 17 (basal pulse rate 98).

Operation was advised against. The thyrotoxicosis, while mild, caused enough discomfort to warrant treatment and was brought under control by cautious x-ray irradiation to his thyroid gland. At the same time deep x-ray therapy to the pituitary gland was started. He has so far received three courses, 900 r each, with no change in the ocular condition.

*Summary of Case 4.* A man aged 49, developed mild thyrotoxicosis and severe ophthalmoplegia with exophthalmos at the same time. The mild thyrotoxicosis was easily controlled by x-ray irradiation of the thyroid gland. Deep x-ray therapy to the pituitary gland was employed. There has so far been no improvement of the ophthalmopathy.

**Case 5:** S. H., a white man aged 38, presented himself with the following history. Two years previously he had noticed bulging of the eyes. At the same time he began tiring very easily, and was drowsy most of the time. His skin became dry and perspiration ceased almost entirely. The eyes became very painful, the lids and the conjunctivae became swollen, and an ulcer of the cornea developed. He was seen elsewhere, and a BMR of minus 40 was found. He received thyroid medication and x-ray irradiation of the pituitary gland. His condition improved considerably.

When seen by us two years after the onset he was still taking desiccated thyroid tablets. He had moderate exophthalmos, but no swelling of lids or conjunctivae. Ocular movements appeared entirely free. His skin was moist and he was alert. He was advised to continue treatment under the care and supervision of the physician who had been treating him.

*Summary of Case 5.* A man aged 38 developed a severe ophthalmopathy with exophthalmos, swelling of lids and conjunctivae and ulceration of the cornea simultaneously with the onset of spontaneous myxedema. There was no indication from the history that the myxedema had been preceded by thyrotoxicosis. Treatment with desiccated thyroid and x-ray of the pituitary gland resulted in almost complete recovery, with only a slight residual exophthalmos.

## DISCUSSION

The problem of "hyperophthalmopathic Graves' disease" has recently been reviewed by Means (9, 10). Briefly there is much evidence that exophthalmos in thyrotoxicosis is not caused by excessive amounts of circulating

thyroid hormone. In animals all manifestations of thyrotoxicosis except exophthalmos can be produced with thyroid hormone or with thyroxin. Exophthalmos, however, can be produced by administration of pituitary extract containing thyrotropic hormone and the exophthalmos is more readily induced and more severe in thyroidectomized animals receiving pituitary extracts (4, 5, 8, 19). In humans the exophthalmos of Graves' disease increases in more than 50 per cent of all cases following thyroidectomy as was shown by actual measurements of the exophthalmos (2, 21). The fallacious impression of improvement of exophthalmos in such cases is due to the "stare" being diminished when thyrotoxicosis is under control.

The conclusion from these observations is that the exophthalmos is caused by a pituitary factor, either the thyrotropic hormone or some other factor as yet unknown. Release of the anterior pituitary following thyroidectomy ("taking the brakes off") leads to an increased secretion of thyrotropic hormone as has been shown by assay of thyrotropic hormone in the urine (6), and thereby causes increase in exophthalmos.

The pathological changes in the orbital tissues (fat, muscle) have been extensively studied in humans (12, 13, 18) and experimental animals (18). Edema, swelling of the extrinsic muscles, and increase of the retrobulbar fat are the main findings.

There still is some difference of opinion as to whether this mechanism holds true for all types of exophthalmos. Mulvany (12) has proposed a dualistic theory contrasting the exophthalmos in ordinary Graves' disease ("thyrotoxic exophthalmos") with that in cases without thyrotoxicosis ("thyrotropic exophthalmos"). The former, the thyrotoxic exophthalmos, Mulvany explains by action of the thyroid hormone on the smooth muscles of the orbit as well as on the extraocular muscles, reviving in somewhat modified form the old theories attributing exophthalmos to the sympathicomimetic action of thyroid hormone.

On the other hand, Means (9, 10) and others strongly advocate a unitarian theory of exophthalmos. There are all transitions from "classical" Graves' disease with severe thyrotoxicosis and exophthalmos through euthyroid to hypothyroid and myxedematous conditions with severe ophthalmopathy. Nor is the "type" of exophthalmos constant or fixed in any one patient, and what started out as "classical" Graves' disease may later become "hyperophthalmopathic."

Mulvany has based his dualistic theory largely upon morphological studies of the intraorbital tissues. Rundle and Wilson (18) on the basis of histological studies of a similar material find evidence of a unitarian genesis of all exophthalmos, "classical" and "hyperophthalmopathic." It is per-



haps possible that what Mulvany interprets as two different processes may be different stages and different degrees of the same condition.

The cases presented in this paper fully support the theory that the exophthalmos is not the direct result of the hyperthyroidism, viz., of the excess of circulating thyroid hormone. They do not disprove the existence of a second type (thyrotoxic) exophthalmos, but we find the evidence for such occurrence unconvincing in the light of facts briefly mentioned above and extensively discussed by Means (9, 10). In two of the cases (No. 1, 2) the ophthalmopathy became much more severe following subtotal thyroidectomy, and in one (Case 5) the ophthalmopathy developed simultaneously with the onset of myxedema, which apparently had not been preceded by thyrotoxicosis, and was, therefore, not the result of a "burnt out" toxic goiter.

Case 3 of our series is particularly instructive. This patient's disease started with severe ophthalmic manifestations (exophthalmos, paresis of extrinsic muscles, but no swelling of lids) with almost no hyperthyroidism. The BMR rose, and the serum cholesterol decreased at a time when the ophthalmopathy was considerably improved under treatment. This case warrants further discussion from another standpoint. The question presented itself as to whether prolonged administration of large doses of diethylstilbestrol had caused the rise in BMR as has been described in the rat (7). On the other hand the claim has been made that estrogenic hormone decreases thyroid function and attempts have even been made to use estrogens in the treatment of thyrotoxicosis (3). However, we have not been able to demonstrate an inhibitory effect of estrogen upon the uptake of radioactive iodine by the thyroid (14). The fact that the BMR remained elevated after cessation of stilbestrol treatment though on a lower level than was present during administration of the estrogen, and responded promptly and typically to iodine medication makes us believe that this patient went into spontaneous exacerbation of thyrotoxicosis. This exacerbation was in all probability independent of any therapy. The unusual feature presented by this patient, namely hyperthyroidism (as evidenced by consistently high BMR and low serum cholesterol and typical response to iodine administration) in the absence of any clinical manifestations is difficult to explain on the basis of present knowledge.

### Metabolic response to thyroid and thyroxin

Case 1 showed a surprisingly slow and inadequate response to orally administered thyroid extract. It is known (21) that the metabolic response to thyroid administration depends on the level of the BMR, and not on the presence or absence of the thyroid gland. Evidently there must be ex-

trathyroidal factors of utilization or destruction of the administered hormone as has been recently discussed by Danowski et al. (1).

In order to exclude the uncontrollable factor of absorption of orally administered thyroid material, it was decided to study the reaction to intravenous injection of crystalline thyroxin. This was carried out at a time when the BMR had finally risen to between 0 and  $-2$ . As can be seen from Figure 3 there was no rise of BMR after the thyroxin injection. For comparison the response to a smaller intravenous dose of thyroxin (10 mg.) at two levels of BMR is charted in this figure, the latter two curves taken from Thompson's paper (22). It must be concluded from these observations that either in this case the oxidative processes were peculiarly insensitive to thyroxin, or that the injected thyroxin was inactivated or destroyed by some antagonistic agent. No definite answer is possible at this time to the question raised by the observations. A peculiar insensitivity to administered thyroid occurs in some humans (17) and apparently quite regularly in the dog (1). Rawson et al. have shown that thyrotropic hormone is inactivated *in vitro* by thyroid tissue (15). There is no information as to whether this inactivation is reciprocal, as might be the case if the thyrotropic hormone would combine in some way with the thyroid hormone. Should this hypothesis be verified it would explain the poor reaction to thyroxin in a condition in which an excessive thyrotropin secretion is supposed to exist. Case 2 revealed a similar behavior towards administration of thyroid material, but it was less marked than in case 1.

### Prevention and Treatment

Our cases illustrate the importance of avoiding thyroidectomy in potential cases of severe ophthalmopathy. This has been stressed by a number of authors but is unfortunately not generally recognized in practice. The decision against operation is easy in cases of ophthalmopathy with normal thyroid function, or with hypothyroidism (Case 5), or with a mild borderline thyrotoxicosis (Cases 3 and 4) contrasting with the severe ophthalmopathy which seems "out of proportion." The decision may not be simple in cases in which severe ophthalmopathy is associated with severe thyrotoxic manifestations. This was probably the situation presented by our cases 1 and 2, both of whom we saw only after operation had been performed elsewhere. There may not be any simple and unequivocal warning sign in such cases. It has been suggested that an unusually low BMR resulting from iodine medication is a danger sign. Whereas the BMR under medication with iodine usually levels off at more or less hyperthyroid levels, and only in mild cases is normalized, there are cases in which values as low as minus 20 have been observed, and these have been considered as

potential "malignant exophthalmos" cases (5). We are unable to judge our two postoperative cases from this angle because, according to the reports received from the respective hospitals, no attempt had been made pre-operatively to determine the lowest BMR obtainable, or in other words to wait for the "leveling off" which is the only reliable indicator of the maximal iodine effect obtainable in each case. However, there are cases on record of severe ophthalmopathy developing following thyroidectomy in which the BMR did not drop to unusually low values (11).

Prevention of this condition depends on judicious evaluation of each case. Severe ophthalmopathy out of proportion to the thyrotoxic manifestations is a clear contraindication to thyroidectomy. Great care must be exercised in cases in which the disproportion is not so evident because of severe thyrotoxicosis. The effect of iodine should be studied carefully, and medication continued to the lowest obtainable BMR value. In case of the slightest doubt, operation should be decided against. This is particularly true in view of the availability of other methods of treatment of Graves' disease. A discussion of the relative merits of x-ray therapy, therapy with antithyroid drugs (thiouracil, etc.), or perhaps with radioactive iodine, are beyond the scope of this paper. These methods of treatment appear to be less dangerous to the eyes perhaps because the suppression of thyroid function is more gradual than that accomplished by operation. The antithyroid drugs would seem to offer the additional advantage of complete reversibility of the antithyroid effect, permitting one to set the thyroid function at any desired and tolerated level.

The emergency treatment of the ophthalmopathy is that of protection of the eyes by local measures, followed by decompressing operations (see case 1). The rationale for this operative treatment is the recognition of edema, and increased ("hypertrophic") fat of the retrobulbar structures as the anatomical basis of the ophthalmopathy. Medical treatment, alone in cases not presenting immediate danger, or following surgical emergency measures, is directed towards suppressing excessive secretion of the anterior lobe of the pituitary gland. Large doses of estrogenic hormones, both natural and synthetic, are known to suppress pituitary function; at lower dosage levels, specifically the follicle-stimulating hormone secretion, in larger doses over longer periods of time, the secretion of other pituitary hormones also is inhibited. Suppression of the thyrotropic hormone by this "shotgun action" (Salter) is attempted in treating the ophthalmopathy with estrogens. The effect of thyroid medication on the pituitary gland is primarily that of suppression of the thyrotropic hormone. In the light of recent experiments, thyroid hormone may perhaps also act by inactivating (neutralizing) thyrotropic hormone peripherally (15). Thyroid medication

would then be the procedure of choice at least in the euthyroid or hypothyroid cases (our cases 1, 2, 5). Whether it can be safely given over long periods of time, and in sufficiently high doses in cases of thyrotoxicosis is as yet uncertain. Rienhoff (16) prepared thyrotoxic patients for operation successfully with desiccated thyroid, producing involution of the gland similar to that obtained with iodine. The patients tolerated thyroid medication well. We have thus far decided not to use thyroid medication in the two thyrotoxic cases. X-ray therapy of the pituitary gland, of course, attempts direct suppression of the overactive gland. It is used very commonly but almost always in conjunction with one of the two hormonal methods outlined above. In the one case in which we have used it alone the result was not satisfactory. From a theoretical standpoint one might question the radiosensitivity of nontumorous, albeit hyperfunctioning pituitary tissue.

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# VIRILIZING OVARIAN TUMORS\*

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THE purpose of the present paper is to describe the clinical, histological and hormonal findings in a case of a diffuse arrhenoblastoma, one which, in accordance with ideas hitherto, will be regarded as an "adrenal-like" ovarian tumor; furthermore, to examine whether it is possible, from the cases in the literature, to find transitions in the symptomology between typical arrhenoblastomata and "adrenal-like" ovarian tumors, thus forming a parallel to Teilum's histological studies. A summary will then be given of the available hormone studies, and mention will be made of certain characteristic findings in cases of virilizing ovarian tumors.

Virilizing ovarian tumors are rather uncommon, but very interesting because they are functioning endocrine tumors. There is a great diversity of opinion on the histogenesis and classification of these tumors; little is known of their hormonal relations, which seem to embody a number of problems.

Usually a distinction is made between two different virilizing ovarian tumors: arrhenoblastoma and "adrenal-like" ovarian tumor.

The arrhenoblastomata received their name from R. Meyer (17) in 1930. His great merit was that he was able to show the connection between types which histologically were far apart and thereby put them together into one form of tumor. He divided the arrhenoblastomata into three groups according to the degree of differentiation. He considered the testicular adenoma (adenoma tubulare), which was first described by Pick (21) in 1905, as the most differentiated. An intermediate group comprises histologically both tubular and diffuse areas. This forms the transition to the third group, the least differentiated form, which has a diffuse, "sarcoma-like" structure. The two latter groups sometimes contain more or less numerous epitheloid, often lipoid-containing cells. Owing to their greatly varying appearance it is sometimes difficult to make a purely morphological division from feminizing tumors of the granulosa cell group (diffuse type). The hormonal effect is greatest in the diffuse forms and is rarely observed in the purely tubular adenoma. We have no definite knowledge of the hormone-producing cells.

About sixty cases of arrhenoblastoma have been described. "Adrenal-like" tumors are still more uncommon. (The one described below is No. 19.) They consist of large, light-colored, often lipoid-containing, epitheloid cells. There is no tubular structure to be seen, for which reason the tumor as-

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sumes a uniformly diffuse appearance. Hitherto the form has proved to be virilizing. The nature and genesis of these tumors have given rise to considerable disagreement, for which reason they are known by many different names: adrenal-cell tumors, adrenal cell rest tumors, hypernephroma in the ovary, luteoma, masculinovoblastoma, etc. Morphologically the cells are very similar to both lutein cells and cells in the adrenal cortex.

One aspect of the disagreement has been as to whether the tumor comes from aberrant adrenal cortex or is a luteinized granulosa cell tumor or a true lutein cell tumor. Schiller (24), e.g., rejects the theory of luteinized granulosa cell tumors, also for morphological reasons. He admits only two possibilities: they are either tumors of adrenal tissue or tumors of a form arising out of a one-sided development of interstitial cells corresponding to Leydig cells in the testicles. As stated, such epitheloid cells occur in larger or smaller numbers in typical arrhenoblastomata too. One-sided development of mesenchymal cells is comprehensible according to Fischel's theory (6), which in the testicles traces all epithelial elements in the ducts, except spermatogonia, from a common mesenchyme nucleus.

Schiller arrives at the conclusion that the question is one of aberrant adrenal cortex, as he is unable to find the histological transition between arrhenoblastomata of ordinary type and "luteomata." The commonest opinion now is that they are tumors emanating from adrenal cortex.

With his studies of homologous tumors in ovary and testis, Teilum (28-30) has thrown entirely new light upon these problems. He has succeeded in demonstrating tumors in testicles (called androblastoma series) showing total morphological congruence with ovarian arrhenoblastomata in all the various forms of differentiation, and also in demonstrating gradual transitional phases between these forms. In addition, by demonstrating transitional phases in typical arrhenoblastomata to parts with uniform (diffuse, alveolar or tubular) structure—corresponding to the structure of virilizing ovarian tumors which hitherto have been regarded as "adrenal-like"—Teilum has extended the arrhenoblastoma series so as to comprise these diffuse types as well. The material seems convincing. It may be taken as an affirmation of R. Meyer's doctrine regarding ovarian arrhenoblastomata, that these tumors all emanate from a special, testicular anlage. It means furthermore that "adrenal-like" ovarian tumors are most naturally to be regarded as a variant of a diffuse arrhenoblastoma, some of which show differentiation in the direction of "interstitial cell tumors" in the testes, and that the idea of adrenal tumors in the ovaries may be dropped.

We may imagine the entire series of androblastomata as tumors growing from a mutually potential male mesenchyme nucleus with differentiation in various directions (tubules, interstitial cells) and with various degrees

of differentiation. As regards their direction of differentiation, testicular adenomata and "adrenal-like" ovarian tumors may be regarded as polar opposites within the androblastoma series.

Among the diffuse forms we may possibly take it for granted that the tumors are differentiated in various directions. Among "adrenal-like" tumors the majority contain lipoids. Possibly these signify a differentiation in a direction other than that taken by the few non-lipoid containing, "adrenal-like" tumors, so that those containing lipoids need not necessarily be considered as having evolved out of a continued development of those not containing lipoids.

Kepler and co-workers (11) consider, on the basis of 14 cases of "adrenal-like" ovarian tumors (thirteen from the literature and one of their own), that clinically it is possible to distinguish between arrhenoblastomata and "adrenal-like" tumors. Apart from virilism, the latter are associated with many of the signs characterizing Cushing's syndrome (hypertension, hyperglobulia, striae caeruleae, diabetes), whereas the arrhenoblastomata cause pure virilism. They leave open the question of how the tumors are to be regarded histogenetically.

#### CASE RECORD

S.M.H., an unmarried woman, 50 years old, was transferred here from the Neurological Department of the Rigshospital with the diagnosis: endocrine affection; meningo-encephalitis hypothalamus region.

There is no record of endocrine affection in the family. The parents were not related.

Menses from 16th year were always regular, lasting three or four days every fourth week. Menstruation ceased at 44th year. At the same time she began to lose her hair and in less than a year was almost completely bald; has since worn a wig. When she was 46-47 years old a vigorous hair growth began to appear on her chin, cheeks, chest, abdomen and back; the baldness persisted. Shortly afterwards there was a sudden increase of weight, about 5 kg. (11 lb.) in a month. At the same time the skin of her face, chest and back became very greasy with large pores. She had always had slight acne, but this did not spread. She was afflicted with strong and protracted flushes, during which her face and neck turned bluish-red and her head felt as if it would burst. The attacks lasted about two hours. During the last two years she had noticed a growth of the clitoris, but no change in the breasts. At about the age of 45 her voice became husky, unclear, and later it "broke" like that of boy at pubescence. As she had to talk a great deal in her occupation, the voice change was a considerable embarrassment and she had to take lessons in voice control.

The patient was of a diffident nature and shy of making contact with people. From her 44th year she withdrew more into herself, became depressed, strangely split, felt tired, got out of balance. From previously being on the best of terms with others, she became hot-tempered, excitable, abusive and quarreled with people. There came a change in erotic attraction; she began to take notice of young girls and their appearance.

During the four years preceding admittance to the hospital she had been given energetic treatment. For about a year she was given a course of di-iodothyrosine tablets, as her doctor thought that her baldness was due to increased metabolism. After her sudden



increase in weight—which set in a month after she had ceased taking the di-iodothyro-sine tablets—she was treated with tablets of glandular thyreoidine, 1 tablet three times daily for three years. In the same period she was periodically treated with estradiol-monobenzoate. She had diathermy treatments for the hypertrichosis but without success.

Four or five months prior to coming to the Steno Memorial Hospital she was run over. She sustained a blow in the back, but no head trauma. Thereafter she suffered from dizziness and her memory began to fail. She had no headache. On account of her vertigo she was admitted to the Neurological Department of the Rigshospital in November, 1944.

Here nothing definitely abnormal was found neurologically, and she was transferred to the Steno Memorial Hospital for further observation for endocrine disease.

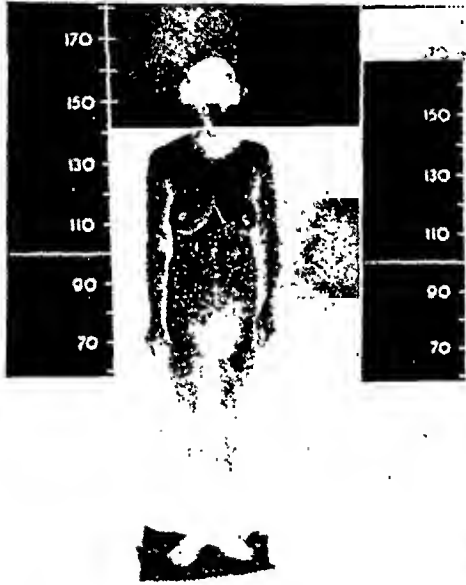


FIG. 1



FIG. 2



FIG. 3

Patient with virilism, before operation.

We found the following: weight and height normal; appearance strongly virilized; no head hair except at temples and nape (her baldness exactly like seborrhoeic baldness in a man); very vigorous hair growth on cheeks, lips, chin, neck, back, chest, abdomen (especially in the median line), lower legs and forearms; a less vigorous growth on thighs and upper arms. The hairs everywhere were long, rather thin and soft.

The skin was very seborrhoeic and large-pored, especially on the face, but also on the other sites of seborrhoea. The skin on the face and neck was dark red, as if it were more weatherbeaten than elsewhere. There were no acne pimples, ecchymosis or perniosis of the skin; no striae caeruleae. Her muscular relief was prominent, especially on the shoulders, upper arms and thighs. The subcutaneous veins of the forearms were very conspicuous. Her breasts seemed quite normal for her age. Her voice was dark, husky, but not particularly virile (voice production courses!). The patient was restrained, depressed, touchy.

Gynecological examination by two different gynecologists at an interval of a month showed: labia majora natural; clitoris considerably enlarged almost to the size of the end of a man's middle finger; hymen adest, speculum could not be introduced; uterus of nor-

mal size, freely motile. At the left lateral margin, almost up at the tuba corner, an intumescence (fibroma) the size of a large nut could be felt. Nothing definite could be felt at the sides of the uterus. Suggested: exploration under narcosis, the exploration find not being quite certain.

The remaining objective, routine examination of the organs revealed nothing abnormal; no tenderness or swelling could be found in the adrenal regions or the abdomen.

The photographs (figs. 1, 2 and 3) show some of the features mentioned. If the medical history were not known, the photographs might be mistaken for those of a man with gynecomasty. Bingel's expression (1) "male potator" is very apt.

Other examinations of the patient<sup>1</sup> showed: height: 162 cm. (64 in.); weight: 58 kg. (128 lb.); blood pressure: 110/60-120/70; urine: no albumin, no sugar.

*Blood:* W.R. neg. S.R. 4 mm., Hgbl. 110-115 per cent, red corpuscles 5.23 millions, white 5880; differential count of white corpuscles normal. Red blood picture natural. Vol. index 1.07, color index 1.10. Serum calcium 9.6-9.9 mg. per cent. Total base: 145.8 m.eq./litre. Serum sodium (1) 149 m.eq./litre, (2) 134 m.eq./litre; serum potassium (1) 5.0 and (2) 5.8 m.eq./litre.

The fasting blood sugar was determined thirty-two times; nine times it was over 100 mg. per cent, once it was 122 mg. per cent. On two different occasions glucose tolerance curves with 70 Gm. glucose took the same course: an increase from 90 to 220 mg. per cent in an hour; the initial value was reached after 2½ hours. Urine sugar tests 1, 2, 2½ and in one test 3½ hours, after commencing the glucose load, gave +sugar.

*Standard metabolism:* +33, +14, +17, and +12 per cent of normal.

*Spinal fluid:* Clear; cells 0/3; globulin 1-(2?), albumin 26-27, Pandy +, W.R. —.

*Eye examination:* Limits of visionary field natural; no color-hemianopia; pupil reactions and eye movements natural; ophthalmoscopy natural, papillae especially being natural.

*X-ray:* Skull: Marked diffuse symmetrical thickening and sclerosis of squama frontalis (hyperostosis cranialis interna). At the thickest spot the cranial wall measures 14 mm.; sella turcica natural.

*Spine:* No osteoporosis.

Urinary organs before and after injection of Uroselectan B: nothing abnormal, particularly in the adrenal regions.

TABLE 1. THE EXCRETION OF ANDROGEN, 17-KETOSTEROID, ESTROGEN AND GONADOTROPIN IN 24-HOUR URINE BEFORE AND AFTER OPERATION FOR VIRILIZING OVARIAN TUMOR

	Dec. 16, '44	Jan. 5, '45	Jan. 7, '45	Feb. 1, '45	Feb. 15, '45	Mar. 6, '45	Mar. 14, '45	Aug. 24, '45*	Jan. 7, '46
Gonadotropin (m.u.)	<50			<50			<50	>50	
Estrogen (m.u.)	>20 <200			<20			<20	ca. 40	
Androgen (i.u.)	50	65	15		3	8	3	6	3
17-Ketosteroid (mg)	12		17	1.8	3.2	0.1	4.0	8.6	

\* Treated with stilbestrol 0.1 mg. ×3 daily from Apr. 7 to Aug. 21, 1945.

<sup>1</sup> The examinations were made partly at the Rigshospital, partly at the Steno Memorial Hospital.

## HORMONE TESTS OF URINE

All the hormone tests made before and after the operation are shown in Table 1.

The androgen was determined by the cock's comb method with direct application after Fussgänger subsequent to simultaneous hydrolysis and benzole extraction of the urine and dissolving of the evaporated extract in oil. Comparison with dose-response curves. (See Hamburger, et al., 1945 (9)). The determinations were carried out at the Hormone Department (Chief: C. Hamburger, M.D.), State Serum Institute.

The 17-ketosteroids were determined by Callow's method (4). The analyses were made at the Biological Laboratory (Chief: K. Pedersen-Bjergaard, Ph.D.), Leo Chemical Works.

## DIAGNOSTIC CONSIDERATIONS

Judging from the clinical indications, the patient probably has a benign tumor or hyperplasia in an endocrine gland, which presumably is either the hypophysis, the adrenal cortex, or the ovary. Consideration must also be given to the possibility of an affection of the brain in the vicinity of the hypophysis (the spinal fluid analysis). As all the cardinal symptoms of Cushing's disease and of Cushing's syndrome (adiposity, hypertension, striae caeruleae, osteoporosis) are absent, and there are merely a slightly reduced carbohydrate tolerance and hyperostosis cranialis interna as the sole—very diffuse and indefinite—signs of a lesion of the hypophysis, it is improbable that there is a primary affection of the hypophysis. Partly for the same reasons a cerebral lesion is unlikely. The spinal fluid analysis is hard to evaluate, being so isolated with no other abnormal neurological find. It might rather be associated with a possible encephalitis in conjunction with the severe influenza she had when 24 years old; this would then be the only remaining sign of that illness.

As the virilism is almost pure, the differential diagnosis must be between an ovarian tumor and an adrenal cortex tumor, or hyperplasia. Gynecological examination failed to reveal any ovarian tumor. X-ray examination showed no enlargement of the suprarenals. (Perirenal air insufflation was not employed, as it is considered dangerous.) The serum sodium and potassium analyses were normal at the first examination, whereas at the second one sodium was slightly low and potassium somewhat high. This is a change in the same direction as that seen in Addison's disease and opposite to what has been observed in the case of some adrenal cortex tumors. The total base is also low.

*Hormone tests:* An excretion of 65 i.u. of androgen and 17 mg. of ketosteroid in 24 hours at the age of 50 must, after the author's investigations (19) and experience, be described as high for this age, though not necessarily pathologically high. It tells us that the patient may possibly have a functioning tumor, but not where it is situated. To be of diagnostic importance the output of hormone metabolites must be very large. Up to the present, at any rate, they have been found only in cases of adrenal cortex tumors. On the other hand, the fact that hormonal values are normal or only slightly increased does not exclude an adrenal cortex tumor. In the present case the hormonal values agree well with an ovarian tumor, but the investigations here are few as yet. (See later.)

*Treatment*

It was not possible to make a fully exact diagnosis prior to the operation. As there was nothing in the clinical picture as a whole that argued against a virilizing ovarian tumor, the conclusion was that in all probability the patient had a benign ovarian tumor but that a lesion of the adrenal cortex could not be excluded.

Laparotomy was therefore decided upon, with an examination of the ovaries and palpation of the suprarenals from the peritoneal cavity.

Laparotomy was performed on the 24th of January, 1945 by Dr. H. Wulff. He removed a smooth tumor the size of a hen's egg from the right ovary and a fibroma of about the same size from the left margin of the uterus. Digital exploration of the suprarenals from the abdomen showed nothing abnormal, and all other organs were found to be normal.

*Description of preparations:* The right ovary had been transformed into a tumor measuring  $4.5 \times 3.5 \times 2$  cm., weight 22 Gm. The surface was grayish-white, quite smooth, and covered with a thick tunica albuginea. Inside this was a brown-red, somewhat moist tissue. Microscopically there was peripherally a narrow or somewhat broader border of a cellular ovarian stroma with small, slender cells. A few fibrous bodies were embedded in this stroma. Within this stroma zone was a node consisting of narrow or broader trabeculae of closely packed roundish or polygonal, medium sized or bigger cells (fig.

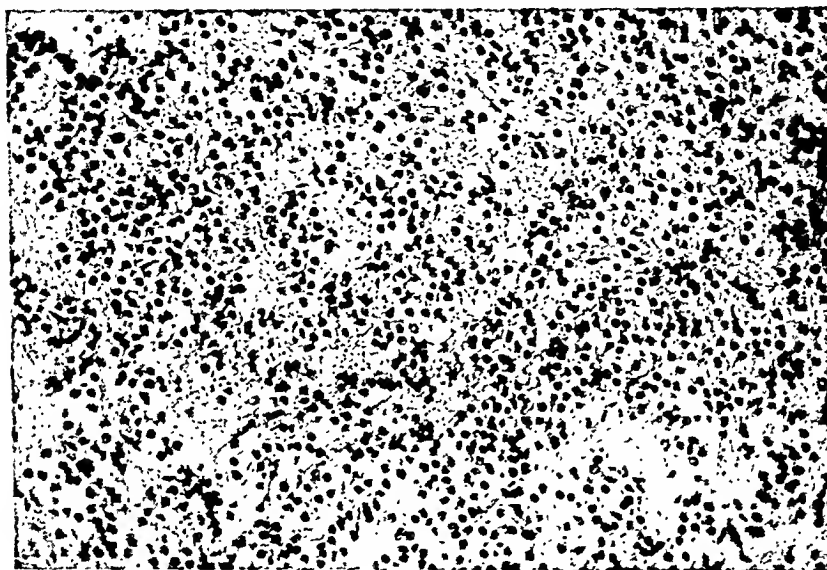


FIG. 4. Section of ovarian tumor: diffuse, no lipoids demonstrable.  
*Arrhenoblastoma diffusum.*

4). The cytoplasm was fine grained, fairly abundant, and in places vacuolized. The nuclei were round, sharply contoured, varying somewhat in size, and with an extremely slender chromatin structure. The nucleoli were often distinct. There were no mitoses. The cell trabeculae were spun with an extremely fine-threaded, richly capillarized, rather hyperemic stroma. An examination of the numerous sections reveals no glandular arrangement of the cells. Fat staining was negative. There was no sign of malignancy.

Thus the histological structure of the tumor agrees with what has previously been regarded as an "adrenal-like" ovarian tumor of the diffuse, non-lipoid containing type. Dr. Teilum, who has been good enough to make a close examination of the histological preparations, considers, after comparison with other gonadal tumors, that this must be a purely diffuse tumor of the androblastoma series with differentiation in the direction of testicular interstitial cell tumor (30).

Ovarian tissue from the left ovary consisted of a cellular stroma with slender cells. In the stroma were a few fibrous bodies and small follicle cysts. There were no inflam-

matory infiltrates. The right tube was microscopically normal. The fibroma showed nothing unusual under the microscope.

*Follow-up examination:* The post-operative course was uncomplicated. The patient was discharged on March 17, 1945, from the Steno Memorial Hospital. Virilism was even then disappearing fast. Menstruation had not returned.

Seven months after the operation the patient was admitted again for control examination. She stated that ten weeks after the operation she had had severe flushes, for which her doctor treated her with stilbestrol benzoate 0.1 mg. three times daily, with good effect on the flushes. After ten weeks of this treatment she had vaginal bleeding for

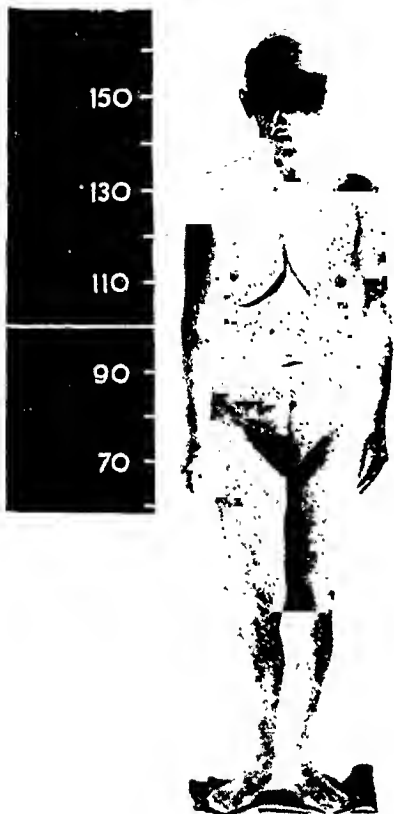


Fig. 5



Fig. 6

Patient 7 months after removal of ovarian tumor.

eight days. This had not recurred. Together with the bleeding there were slight pains in the left fossa iliaca, and they had since recurred now and then.

*Objective examination* (figs. 5 and 6): The patient was still psychasthenic, depressed, restrained, absorbed in her earlier affection. Looked climacteric. She repeated that she had been relieved of great pressure and inner tension.

About her appearance there was now nothing remarkable, but she was slightly aged. She had a feminine effect. The hypertrichosis had gone completely. Beyond the classical normal hair there was only a slight covering on legs, forearms and hands. On her head the hair had grown somewhat but was still very sparse. The skin of the scalp was natural. The seborrhoea had disappeared. The physical contours were more rounded, feminine; the breasts a little more full than before. Patient's weight was unchanged. Speech

and voice were feminine and light. The clitoris was still slightly enlarged, but was much smaller than before treatment.

Other examinations: Weight: 58 kg. (128 lb.); height: 162 cm. (64 in.); blood pressure: 110/75; urine: albumin, sugar.

Blood: S.R. 4 mm., Hgbl.: 100, red blood corpuscles 4.5 millions, white 6360. Serum cholesterol 172 mg. per cent.

Fasting blood sugar determined 20 times varied between 88 and 122 mg. per cent; on nine occasions the value was over 100 mg. per cent, once over 120 mg. per cent. Glucose tolerance curves with 70 Gm. glucose showed an increase from 97 to 220 mg. per cent in 45 minutes. The initial value reached in 2 hours 25 minutes. +S in urine an hour after beginning of load -S 2 and 2½ hours after.

Standard metabolism: 90 and 102 per cent. Hormone excretion: See Table 1.

Spinal fluid: 0/3 cells, albumin >20 <30, globulin >1 <2.

Gynecological examination: Hair now natural. External genitals natural except clitoris, which was as large as half of the end of a little finger, but judged to be only half as large as at last examination. Inspection showed nothing out of the ordinary. Recto-vaginal exploration found the uterus natural; nothing abnormal felt in right side. Left ovary felt about the size of a green walnut, somewhat tender. It was movable (corpus luteum cyst?).

Laryngological examination: The larynx very large; the epiglottis especially. The vocal chords very long, quite livid, with smooth margins. Motility good. The voice is at any rate not too deep and has a very good tone.

X-ray of skull: The thickness and the sclerosis of the frontal bone unaltered.

This time the treatment consisted of encouraging the patient's psyche and zest for work.

Fourteen months after the operation the patient reported by letter that her head hair was not yet natural; she always wore a kerchief over her head. Psychically she was not well, being worried and depressed, but felt considerably relieved since the operation.

## DISCUSSION

Only two features of her virilism remained seven to fourteen months after the operation: insufficient growth of hair on the head and a slightly enlarged clitoris. Since the operation the patient has become climacteric and aged. This is undoubtedly due to the cessation of hormone action. It is not to be expected that her menstruation will return at the age of fifty. The vaginal hemorrhage (and enlargement of the left ovary) would have their most natural explanation in the stilbestrol therapy.

Was this patient's virilism pure, or did she present metabolic changes of the Cushing type? There were changes in the carbohydrate metabolism, standard metabolism and in the number of red corpuscles in the blood.

At the first examination there was a slight but definite change in the glucose tolerance curve in the diabetic direction, and the fasting blood sugar value on the whole was rather high. Seven months after the removal of the tumor this change still persisted. Nevertheless, after the operation there were two changes towards the normal: the maximum on the curve was reached fifteen minutes earlier, and the glucosuria was not so protracted. These changes were so slight that one would scarcely assert that they were due to the removal of the tumor.

Prior to the operation the standard metabolism was +33, +14, +17 and +12 per cent of the normal. Seven months after it, the values were -10 and +2 per cent. From being slightly high the standard metabolism had become normal. As the metabolism can

remain high for four to eight weeks after discontinuing the thyreoidin tablets, and as the metabolism perhaps was already falling before the operation, we cannot definitely credit the lower metabolism to the removal of the tumor. It may have been due to the fact that the effect of the earlier, protracted ingestion of thyreoidin was only gradually ebbing out.

Before the operation the number of red blood corpuscles per c.mm. of blood was 5.23 millions, and seven months after the operation 4.50 millions: a doubtful pathological increase before the operation; a doubtful decrease after it. It is particularly true here that more tests before and after the operation would have made the estimate safer.

The conclusion must be that there were slight metabolic changes, but there is no proof that they were due to the tumor, although we cannot exclude that possibility.

### COMPARISON OF OWN CASE WITH LITERATURE RECORDS

Kepler, et al. (11) reckon 14 cases of "adrenal-like" ovarian tumors described in all. One was also described by I. Mayer (16). Since then there have been instances published by Greene & Lapp (8) (No. 16), R. Luft (13) (No. 17), Burket & Abell (2) (No. 18). The present case is No. 19. More may have been published; literature is not fully obtainable.

From the descriptions and photographs there seems to be complete agreement in the histological picture in Sellheim's (25), Luft's (13), and the present case, and possibly Bingel's (1) case. These four cases have it in common not to have much lipoid and, therefore, in a morphological sense, they take up an exceptional position among the "adrenal-like" ovarian tumors. The color of the tissue is grey to brownish red. Clinically there was also close agreement, with pronounced virilism, perhaps due to the patients' being about the same age, 49, 44 and 30 years (Bingel: 47 years), and the duration of the affection, about five or six years (Bingel: 12 years). With reference to the Cushing syndrome, the only cases that are fairly well examined are Luft's and the author's. In Luft's case there were no metabolic changes; in the present case there were changes, as already stated; but only few and slight; in Sellheim's there was a little adiposity; in Bingel's a distinct Cushing syndrome. Signs of Cushing's syndrome are not always found in patients with "adrenal-like" ovarian tumors.

After comparing several of the published descriptions of cases of arrhenoblastoma and "adrenal-like" ovarian tumor, we must agree with Kepler, et al., (11) that metabolic changes of the Cushing type have been observed only among the "adrenal-like" cases. On the other hand it is typical that practically no patient with arrhenoblastoma has been examined as regards these symptoms. Consequently we may possibly assume that there was no pronounced Cushing syndrome among the arrhenoblastoma patients, though we cannot deny the possibility that there were isolated symptoms of it. All in all there seems to be a difference in the clinical indications of the two forms of tumor, as Kepler, et al., point out. The question is whether this difference is so absolute as these workers believe.

We know nothing as yet as to where the limits must be drawn for the hormonal effects within the androblastoma series. It is more probable that the hormonal effect is combined with the differentiation of (some) cells in a certain direction than with the circumstance that the tumor is diffuse without tubular regions. Mention has been made of the possibility of differentiation in various directions within the diffuse tumors.

Whatever may be the true fact, it will mean clinical support for Teilum's histologically-based theory that "adrenal-like" ovarian tumors belong to the arrhenoblastomata and are not adrenal cortex tumors, if we can find arrhenoblastomata of common type containing tubular regions, with clinical signs of the Cushing syndrome.

I have been unable to find pure cases of this kind described. The nearest are Canelo & Lissner's case (5), and Kraus's (12), to which I would refer interested readers. As the tumors were only discovered postmortem in both cases, we lack the definitive proof of the causal relation between tumor and signs, i.e., the subsidence of signs after the removal of the tumor.

TABLE 2. ANDROGEN AND 17-KETOSTEROID EXCRETION IN 24-HOUR URINE

Tumor	Lipoids in tumor	Author	Pt.'s age	Androgen		17-Ketosteroid	
				Before operation	After operation	Before operation	After operation
Diffuse: "adrenal-like" cells	0	J. Pedersen 1946	50	43 i.u.	5 i.u.	14.5 mg.	3.5 mg.
	0	R. Luft (13) 1944	44			23.8 mg.	
	+	Kepler et al. (11) 1944	16			54.6 mg.	2.6 mg.
	+	Rottino et al. (22) 1939	23	16 i.u.			
Typical arrhenoblastoma (tubules)		Mathiesen (14) 1944	24			4 mg.	2.5 mg.
		Wijzenbeek et al. (32) 1940	28	60 i.u.			
		Kanter et al. (10) 1940	33	51 i.u.	10 i.u.		
		Westman (31) 1939	16	12 i.u.	2 i.u.		

### Hormone Excretion of Virilizing Ovarian Tumors

It is most natural to regard the virilism connected with these tumors as being produced by hormones secreted by their growth. It is surprising to see how few are the tests made for the excretion of hormonal substances. Table 2 shows the investigations made during the past few years. In judging this table it should be borne in mind that the technic in each case was different. Besides, some of the values are averages, others merely single determinations. Finally, the patients are in widely different age groups.



This is a drawback to comparison in the androgen series too, where the results are given in international units.

In those cases where an analysis was made the chorionic gonadotropin excretion was normal, as was that of estrogen. Saphir & Parker (23), however, found an increased quantity of estrogen, about 5000 m.u./1 urine, which subsided after the tumor was removed. In Maxwell's case (15), a 67 year old woman, there was up to 40 m.u. estrogen in the 24 hour urine. The hormone content in tumors has several times been determined (e.g. Wijsenbeek & Plate (32), Kanter & Klawans (10)) and has always been very low.

The few hormone investigations available show that the excretion of steroids in the urine is unexpectedly low considering the marked virilism. The values vary from the normal to the low pathological level. This was already observed by v. Szathmáry (27), the first to examine an arrhenoblastoma for hormones. There may be several reasons for the low excretion, for example, steroids not demonstrable by the methods applied. It is theoretically possible that the relatively low values are fortuitous (the few investigations). The high excretion of estrogen in a few cases makes it presumable that the excretion of steroids is just as variable as with virilizing adrenal cortex tumors.

For the present, these hormone analyses are of only limited value to diagnosis. Definitely increased values argue the presence of a virilizing tumor; normal values do not argue against it; the analysis provides no indication as to the origin (ovary, adrenal cortex, aberrant adrenal cortex). No matter what the value of the excretion of androgen and ketosteroid may be prior to the operation, it seems to fall afterwards, so that it may be of assistance to the diagnosis of a recurrence to follow the excretion of hormones after the operation. In the course of time no small number of these tumors have proved to be malignant, often with a late recurrence (Rottino & McGrath (22), Burket & Abell (2), I. Mayer (16), Kepler, et al., (11)).

Investigations so far are too few to demonstrate any difference in the hormone excretion in cases of diffuse arrhenoblastomata ("adrenal-like" tumors) and arrhenoblastomata of the usual mixed type, and any correlation between hormone excretion and clinical symptoms. As with adrenal cortex tumors, we lack clinically employable methods for the specific demonstration of the various steroids. Until such methods become available, it is advisable to determine both the androgens and the 17-ketosteroids, as there need not necessarily be any parallel between them.

Two curious features are common to gonadal tumors of the androblastoma series and adrenal cortex tumors: they are more frequent in females than in males, and, when they act upon the sexual character, they have a feminizing influence on males and a virilizing effect on females. Why?

*Changes in the Endocrine Organs*

One of the observations made on our patient was hyperostosis cranialis interna. Four other authors report on changes in the craniogram which may be interpreted as hyperostoses (Novak & Wallis (18), Rottino & McGrath (22), I. Mayer (16) and R. Luft (13)). All these patients had tumors of the "adrenal-like" type. Thus five hyperostoses have been found among about twenty (not all of which were examined for it). If this frequency is correct, it is considerably higher than in the normal population (Pedersen (20)). It seems convincing that in two of the five cases the hyperostosis occurred at the ages of 23 and 32, an age class in which hyperostosis is rare. In three of the cases, the hyperostosis was of the nodular frontalis interna type; in the other two (Novak & Wallis (18) and the author's case) it was hyperostosis intertabularis (20). We do not know the etiology or pathogenesis of hyperostosis, but it is usually regarded as a somewhat diffuse endocrine stigma, indicating changes in the pituitary.

We are unable to decide whether it is of clinical importance that hyperostosis was found only in patients with "adrenal-like" ovarian tumors; only very few of those with arrhenoblastoma of the usual type were x-rayed.

It is also possible to show changes in other endocrine organs. Struma is not uncommon (Geisler (7), Strassmann (26), Wijsenbeek & Plate (32), Bingel (1), Novak & Wallis (18) etc.), and it occurs in both forms of virilizing ovarian tumor.

Fibroma uteri is also frequent in cases of "adrenal-like" ovarian tumors; it occurs in Bingel's (1), Sellheim's (25), Maxwell's (15), Rottino & McGrath's (22) (case 2), Burket & Abell's (2), the author's, and possibly in R. Luft's (13) cases, i.e., in six or seven out of about twenty. A similar frequency is reported for arrhenoblastomata of the usual type (Büttner (3)).

Very few postmortem examinations have been made of patients with virilizing ovarian tumors, and hitherto they have not presented any particularly characteristic changes in the other endocrine organs. In all probability, the explanation of the Cushing syndrome is the secondary action of the tumor product upon other endocrine organs, especially the pituitary.

## SUMMARY

The author reports on a case of virilizing, diffuse, non-lipoid containing, "adrenal-like" ovarian tumor in a 50-year-old woman. The tumor could not be palpated by gynecological examination. This is the nineteenth case of virilizing "adrenal-like" ovarian tumor recorded in the literature.

Teilum has demonstrated that histologically there are transitional forms between arrhenoblastomata of the usual type with tubular areas and the "adrenal-like" ovarian tumors, for which reason the latter should be regarded as a variant of an arrhenoblastoma diffusum and not as an adrenal

cortex tumor. Kepler, et al., consider that patients with "adrenal-like" ovarian tumors differ from patients with the usual arrhenoblastoma by having more or fewer symptoms of the Cushing syndrome. The author endeavors to find transitions in the clinical signs of patients with arrhenoblastoma of the usual type and patients with "adrenal-like" tumors, in parallel with the transitions which Teilum has shown histologically. No convincing cases were found, presumably because very few patients with arrhenoblastoma of the usual type have been examined clinically for the Cushing syndrome.

Prior to the operation the present patient excreted an average of 45 i.u. androgen and 14.5 mg. 17-ketosteroid in the 24-hour urine; after the operation 5 i.u. and 3.5 mg. ketosteroid. A survey is given of the hormonal determinations recorded in the literature. The diagnostic value of the hormone excretion in the urine is small, but it may be of importance to the diagnosis of a recurring tumor.

Hyperostosis cranialis interna is a frequent feature in cases of diffuse arrhenoblastoma, whereas the frequency cannot be investigated for those of arrhenoblastoma of the usual type, as x-rays exist in a few cases only. Fibroma uteri and struma are frequent in both forms of virilizing ovarian tumor.

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# HYPOGONADOTROPIC EUNUCHOIDISM: REPORT OF CASE WITH FAILURE TO RESPOND TO CHORIONIC GONADOTROPIC HORMONE DUE TO ANTIHORMONES

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THE therapeutic efficacy of most of the available gonadotropic hormone preparations is limited by the rapid development of neutralizing substances generally termed antihormones.

Pregnant mare's serum is particularly prone to call forth the development of these antihormones, thereby restricting the possible usefulness of this therapeutic agent to brief periods. On the other hand, there are available preparations of the chorionic gonadotropic hormone from human pregnancy urine which have appeared to be singularly free of this complicating reaction in man. However, it has been possible to produce neutralizing substances against this human chorionic gonadotropic hormone in various species of animals. This subject has been reviewed by Leathem (5) and Thompson (8).

Despite the lack of demonstrable titers of antihormone against chorionic gonadotropic hormone in man, speculation about its existence has been extensive. A pregnancy test based on the concept that in women antihormones would regularly develop against their own chorionic gonadotropic hormone was proposed (2) but has proved unreliable (3). Furthermore, Leathem and Rakoff (6) failed to find any evidence of such antihormones in a group of postpartum women carefully studied. Fraser and coworkers (1) in Albright's group have suggested that antibodies developed to chorionic gonadotropin from human pregnancy urine, in two patients with hypogonadotropic eunuchoidism (Negative FSH) who had previously been treated with this substance. This suggestion was based upon the observation that these patients failed to respond to administration of this material with an increased output of urinary 17-ketosteroids. No studies for such antihormones were reported.

The case to be described presented a similar problem. Antihormone studies were therefore undertaken.

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of inhibiting equine gonadotropin and chorionic gonadotropin but did not influence synapoidin." In some of the patients who received synapoidin alone antihormones did not develop. However, in patients in whom antihormones against synapoidin developed it was noted that the antiserum would not antagonize the action of human pituitary but did inhibit equine gonadotropin and chorionic gonadotropin.

TABLE 1. ASSAYS OF PATIENT'S SERUM FOR CHORIONIC GONADOTROPIN NEUTRALIZING ABILITY

Date	Chorionic Gonadotropin International Units	Serum cc.	Number of Mice	Uterine Weight Aver. mg.	Ovarian Weight Aver. mg.
2- 4-16	6	0	5	80.1	4.0
	6	1.25	4	17.2	1.6
3-13-46	20	0	3	60.4	6.1
	20	0.9	3	42.6	3.4
3- 6-46	20	0	4	52.3	5.7
	20	0.9	4	40.5	3.6
Untreated Controls	0	0	16	14.5	2.1

### SUMMARY

A case is described of a young man with hypogonadotropic eunuchoidism who failed to respond to adequate therapy with the chorionic gonadotropic hormone from human pregnancy urine. This was demonstrated to be due to the presence of antihormones against this gonadotropic hormone.

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# ADDISON'S DISEASE FOLLOWED FOR NINE YEARS: CASE REPORT WITH AUTOPSY

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**B**ECAUSE of the newer methods of treatment, the life of patients with Addison's disease has been extended for several years. The influence this new form of therapy has on longevity, and its effects on body tissues, warrants reporting a case followed for nine years. Furthermore, the fact that this patient's pigmentation underwent marked changes, either spontaneously or because of the therapy, is an additional reason for this report.

This case was previously reported in the October 1940 issue of *Endocrinology* (2), at which time it was summarized as follows:

"A case is reported in a 31 year old male patient who presented all the clinical criteria for the diagnosis of Addison's disease. The patient had been on adrenal cortex extract and sodium chloride, discontinuance of which resulted in an Addisonian crisis. Desoxycorticosterone acetate was given six times a week in 5 mg. doses for two months. Subsequent to this, 600 mg. of desoxycorticosterone acetate, in the form of four pellets of 150 mg. each, were implanted in the abdominal muscles. Marked general improvement, gain in weight, and a substantial increase in blood pressure resulted from this form of therapy. The improvement has been maintained over a period of five months. There has been no noticeable change in the pigmentation of the skin."

An appended footnote to the article stated: "On July 8, (1940), ten months after implantation, he is doing very well without further medication."

At this point it is well to correct an error in the original report. It was stated that there was at that time, July 8, 1940, no noticeable change in the pigmentation of the skin. The photographer made a kodachrome film of the patient on January 8, 1937, six days after admission to the hospital, as he was recovering from the crisis. Also, a regular black and white print was made, reproduction of which is shown in Figure 1. A comparison of the frontal view of Figure 1, made January 8, 1937, with Figure 2, made July 12, 1937 (in kodachrome and published as such in color in *Endocrinology* (2)), shows a marked change in pigment anteriorly.

This change in pigment, marked by its loss in the neck, anterior part

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of the chest, and arms, is readily apparent to anyone. No change is seen in the pigment of the back, Figure 3. My only explanation for such a gross oversight is that the original kodachrome, taken on January 8, 1937, was not nearly as clear in color contrast as those taken on July 12, the latter being the ones used in the original article. Only recently, following the patient's death, when the black and white prints of January 8 and July



FIG. 1. Photograph made January 8, 1937 showing intense pigmentation with vitiligo or leukoderma of hands.

12 were made, did the contrasting pigmentary changes which came to pass during that time register in my mind. It must be said, however, that both the patient and myself were aware of changes which developed later. Be that as it may, this is no excuse for the clinical oversight.

With this note of apology, in order to date the case, it should be stated that he: "Was first seen on January 2, 1937, with the complaint of abnormal pigmentation of the skin, urticaria, nausea and vomiting, and loss of weight. He always had been dark, he said, but had become much darker during the last eight months. His mother stated that his color did not compare with that previous to his illness. Associated with this, he had



FIG. 3

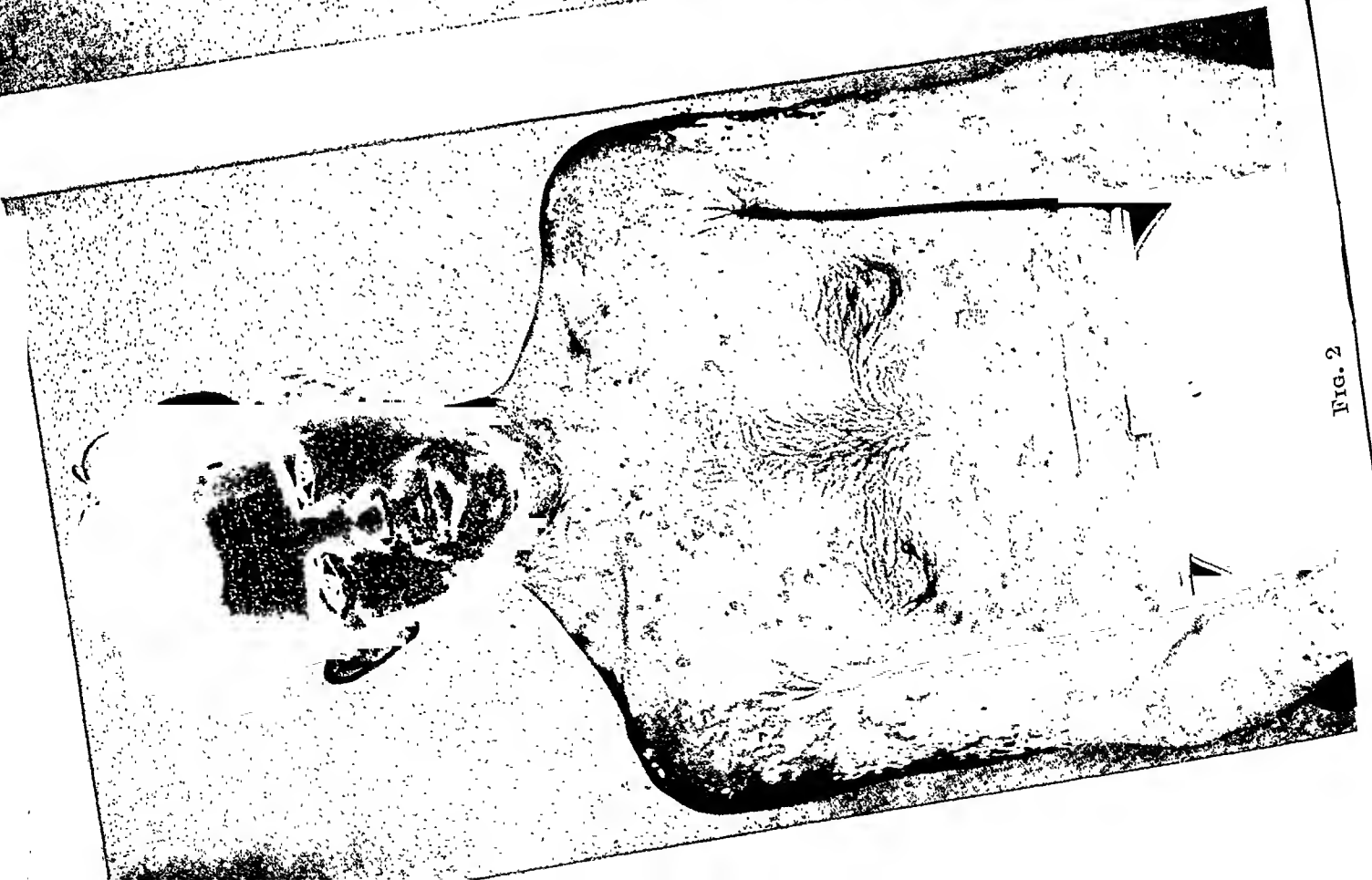


FIG. 2

marked weakness, nausea, and vomiting. The nausea and vomiting had been present for one month, but the weakness had been progressing for eight months"; (since approximately April, 1936).

It is of some importance that a maternal aunt died of Addison's disease, the diagnosis having been verified at autopsy. No tuberculosis was found. After the death of the patient, it was learned that the daughter of a brother (patient's niece) was showing pigmentary changes and was to be examined.

The salient features of this patient's subsequent course are as follows:

Photographs made January 23, 1940, that is, three years after he was first seen, showed no change in pigmentation from those of July 12, 1937 (Figures 4 and 5).

One year after the implantation of the pellets, September 7, 1940, he felt well and his blood pressure was systolic 94, diastolic 60. The blood chlorides were 465 mg. He was taking 2 Gm. of sodium chloride daily, but no other medication. His weight was 62.1 kg. (137 pounds). During the original crisis, he weighed 53.1 kg. (117 pounds). When seen February 10, 1941, it was noted that he had lost weight, 2.3 kg. (5 pounds), and his blood pressure was systolic 70, diastolic 62.

He felt nauseated and weak. The blood chlorides were 350 mg., with the recurrence of symptoms seventeen months after the implantation of 600 mg. of desoxycorticosterone acetate. It was felt that this procedure should be repeated, and on February 12 three pellets of 150 mg. each (450 mg.), were implanted in the left scapular area. Two Gm. of sodium chloride were taken daily.

On March 28 his blood pressure had reached 122/70. There was some edema of the face and ankles, and the salt was discontinued.

On April 7 he said that he felt fine and the edema had subsided.

The pigmentation of the interscapular areas had begun to fade. Colored movies had been taken soon after recovery from the original crisis in 1937, and now, in April 1941, additional ones were made and these showed loss of pigmentation on the anterior chest, arms, and back. The amount of pigmentary loss was marked.

On September 9 it was noted that there was still more fading of pigment on the back. His blood pressure, however, began dropping, being 100/64. On October 2, about eight months after the last implantation, the mother reported by telephone that he was vomiting, was weak, and was not doing

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FIG. 2. Anterior view made July 12, 1937 showing loss of pigment from chest, neck and arms. Urticaria is also present.

FIG. 3. Posterior view made July 12, 1937.

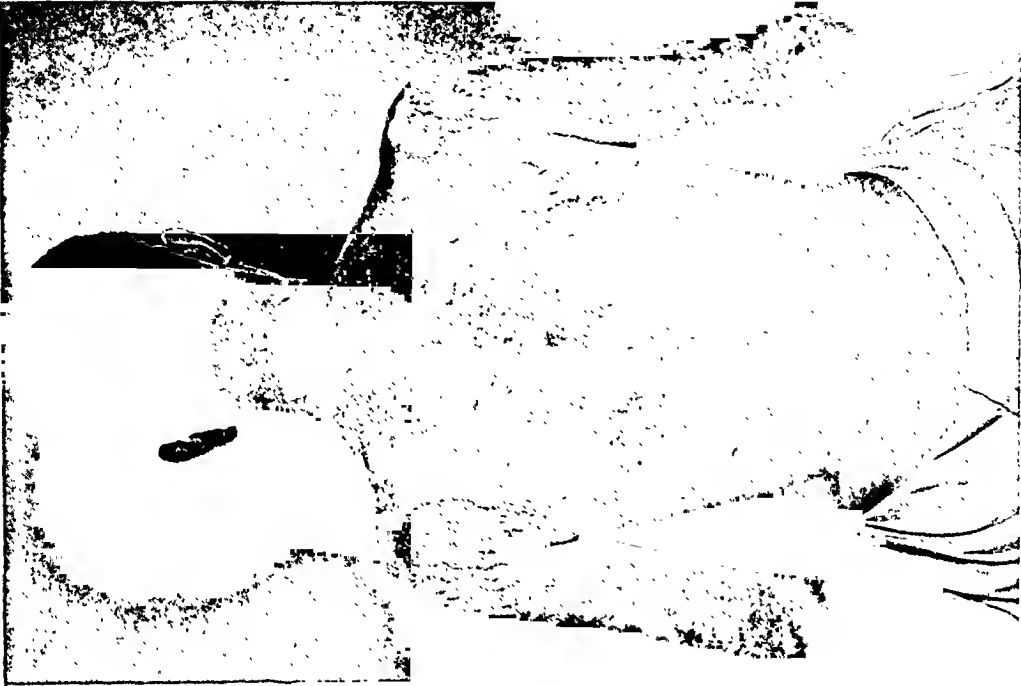


Fig. 5



Fig. 4

Fig. 4. Anterior view January 23, 1940 showing no change in pigment from that of July 12, 1937 (Figure 2).  
Fig. 5. Posterior view made January 23, 1940 showing no change in pigment from that of July 12, 1937 (Figure 3).

well. He was seen the next day, at which time he complained of severe headache, marked asthenia, and vomiting. His blood pressure was 86/66. Five Gm. of salt, and 10 mg. of desoxycorticosterone acetate, were given hypodermically. He was sent into the hospital, and with daily injections of aqueous adrenal cortex extract, desoxycorticosterone acetate, intravenous glucose in saline, sodium chloride, vitamin C, and a low potassium and high sodium diet, he recovered rapidly.

An important finding at this time was a severe anemia. The hemoglobin was 58 per cent, 2,860,000 red cells, and only 2,500 leukocytes. The differential count was 60 per cent polymorphonuclears, and 40 per cent lymphocytes. The chlorides were 429 mg. on the second day after admission, following therapy. He was placed on 5 mg. of desoxycorticosterone acetate daily, 5 Gm. of sodium chloride and .2 (3 grains) of ferrous sulfate four times a day. The latter drug produced watery stools and had to be discontinued, but was resumed one week later with three doses per day. His blood count improved, and on October 24 hemoglobin was 73 per cent, 3,930,000 red cells and 5,500 leukocytes, with a normal differential count.

He was seen at monthly intervals with no marked change in his condition. The blood pressure fluctuated between 100/64 and 124/80.

He did well until June 13, 1942, when he again complained of weakness, vomiting and severe headache. He said the salt caused the headache, and had discontinued it May 1. His blood pressure was 100/62. Another implantation of 525 mg. of desoxycorticosterone acetate pellets was made in the left scapular area on June 15. The total amount of pellets implanted was 1,575 mg.

By June 26 he was once more on the road to recovery with a blood pressure of 124/80. He was taking 200 mg. of vitamin C and .6 (9 grains) of ferrous sulfate per day. His appetite improved.

On November 28, he had a chill, fever and weakness. Five mg. of desoxycorticosterone acetate and 2 Gm. of sodium chloride were given daily.

He progressed well on this medication until June 10, 1943, when on his visit to the office, he stated that he was tired of giving himself hypodermics, and it was decided to use sublingual desoxycorticosterone acetate in propylene glycol. A dosage equal to 3 mg. was given sublingually. In addition, 1 Gm. of sodium chloride was also given.

He developed an upper respiratory infection on October 4, and his blood pressure dropped to 80/48. A double dose of the sublingual medication was given and the sodium chloride increased. He occasionally felt weak and shaky and it was thought that hypoglycemia was present, but no blood sugar studies were made.

His pigment continued to fade and on April 10, 1944, photographs were taken (Figures 6 and 7).

The leukodermic areas around the eyes were especially noticeable, and increased his bizarre appearance. Very little pigment remained anteriorly except on the face and neck. The pigment on the back and arms had also largely disappeared except on the neck and a few areas on the shoulders and arms.

On December 17, 1944 he said that he was weak and his blood pressure was 100/82. He was given, in addition to the sublingual medication, 2 cc. of adrenal cortex extract. He complained that his mouth watered a great deal, and on February 9, 1945 the submaxillary glands were swollen to approximately twice their normal size and salivation was troublesome. The sublingual cortate was stopped, and the adrenal cortex was increased to 4 cc. per day.

By March 2, the salivary-gland swelling had subsided and the salivation disappeared.

On July 16 he returned to a daily dose of 5 mg. desoxycorticosterone acetate. He continued this therapy, and on May 14, 1946 he did not look as well as previously. His blood pressure was 90/64.

In the early part of July he went to a summer cottage on a lake near Detroit, and his family noted that he was acting peculiarly. He made the statement that he was tired of his illness and tired of giving himself "shots." He did not take his medication for two weeks. He became reticent, irritable, and wanted to be alone. On July 23 he became delirious, had a temperature of 101 to 103, and he was sent to Harper Hospital on July 27 in a comatose condition. His blood pressure was 96/70. The hemoglobin was 11.5 Gm., 75 per cent, red cell count 3,550,000, leucocytes 9,900, with a normal differential count. Fasting sugar was 32 mg. and nitrogen 48 mg.

Intravenous glucose, 10 cc. adrenal cortex extract every 4 hrs., and 25 mg. desoxycorticosterone acetate, did not bring him out of the crisis. He remained comatose and his temperature rose rapidly to 106. He became pulseless. His blood pressure could not be obtained. He died July 28, at 3:00 p.m., the day after admission.

The autopsy was performed one hour later.\*

**External examination:** The body was that of a 41 year old white man, moderately well-developed and nourished, about five feet, seven inches tall and weighing about 130 pounds. The hair was thick and black. The face and neck were deeply pigmented with dark tan coloration, except for

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\* My thanks are due Dr. Plinn F. Morse and Dr. Herbert W. Devine for the reports on the autopsy.



FIG. 6



FIG. 7

FIG. 6. Anterior view made April 10, 1944 showing further loss of pigment from arms. Vitiligo around eyes is well shown.

FIG. 7. Posterior view made April 10, 1944 showing marked loss of pigment from back, arms and neck.



small areas about both eyes and the sides of the nose. The eyes were equally dilated. The nose and mouth were normal. The teeth were in fair repair. No pigmentation was seen in the mucous membranes. The ears were thickened and quite rigid in character. The pinnae felt as though they were partially ossified. The thyroid was not palpable in the neck. The neck appeared normal except for the previously described pigmentation. The chest was moderately well developed and resonant bilaterally. There was a one-inch-long scar in the left abdominal region, slightly above the umbilicus and lateral to it. This was the site of implantation of desoxycorticosterone. The upper extremity showed freckles of deep tan pigmentation, while the rest of the body showed marked pallor. There was no evidence of old or recent injury to the extremity. Rigor mortis was slight and livor mortis only moderate.

**Main incision:** The body was opened by the usual Y incision. There was about 1 cm. of white panniculus. The tissues appeared to be dry. The musculature was well developed. There was no fluid in the abdominal cavity. The peritoneum was normal and the organs were in their normal anatomic relations.

The sternum was removed by cutting the costal cartilages. The pleural cavities were dry. No adhesions between parietal and costal pleura were present. The pericardium was opened *in situ*, revealing a small heart. There was about 30 cc. of clear yellow fluid in the sac, which itself was normal and shiny.

The pulmonary artery was opened *in situ* revealing no clot or thrombus. The great vessels of the neck were ligated and the thoracic structure removed *en masse*. The right lung weighed 430 Gm.; the left 400 Gm. They were blue-gray in color and were pneumatized. Both floated well in water. The bases of both lungs showed moderate hypostatic congestion, and on section were wet. On compression in the larger bronchioles, thick yellow secretion could be expressed. There were small calcified glands in both hili and at the left apex there was a fibrotic scar without calcification. The trachea and major bronchi contained a small amount of thick, yellow secretion. The mucous membranes were normal throughout. The aorta showed no atherosclerosis. The coronary arteries were patent at their origin and normal throughout. The aortic valves were normal; the heart was quite small, weighing 210 Gm. The myocardium appeared normal. The ventricles and auricles showed mild postmortem clot, but otherwise showed no abnormality. The tricuspid, mitral, and pulmonary valves were normal. The esophagus showed no abnormality. The organs of the abdomen were in their normal anatomic relation. The liver was small and light red in color; it weighed 1,350 Gm. externally, and on section it showed

normal architecture. The diaphragm was intact. The gall bladder was long and pendulous. It held about 50 cc. of brown bile and contained numerous small pigmented (sand) granules. The mucosa appeared normal. The spleen weighed 240 Gm. and was slate blue in color. On section it showed normal architecture. The pancreas was small and weighed only 50 Gm. On section it showed normal pink-gray lobulated tissue, and the ducts and vessels were of normal consistency. The adrenals were absent, even after extensive dissection above the kidneys down as far as the pelvis. No demonstrable adrenal tissue could be found, even after the perinephritic fat was minutely sectioned grossly. Accessory adrenal tissue was hunted for down the abdominal aorta and along the spermatic vessels, but none was found. The perinephritic tissue was placed in formalin for further investigation. The kidneys were small, the right weighing 70 Gm., the left 80 Gm. They were slightly lobulated, and the left kidney had an old, well-healed infarct in the inferior pole. The capsule was stripped with moderate difficulty. The cortex was thin and petechial; hemorrhages were demonstrated in the left pelvis. The ureters were normal. The bladder was opened *in situ* and showed normal structure. The prostate was small and normal. The stomach was opened, showing postmortem autolysis but no other change. The duodenum, small and large bowel, were normal throughout. The thyroid was dissected out, and the lobes were small and light pink in color. The testes were removed. They also were small but normal in structure.

**Head:** The scalp was incised in the usual fashion and the calvarium removed. The dura was normal. The piaarachnoid was normal. The brain weighed 1,535 Gm., and showed no abnormality externally. The lateral ventricles were opened and were normal. The cerebral vessels showed minimal or no evidence of sclerosis. The brain was placed in fixing solution for further study.

#### Gross anatomic diagnosis:

1. Absence of both adrenals grossly.
2. Extensive pigmentary changes of the skin of the face, neck, shoulders and arms.
3. Cholelithiasis.
4. Small kidneys with fetal type lobulation, and an old, healed infarct of the left kidney.

#### Microscopic diagnosis:

1. The pituitary showed diffuse fibrosis of the whole anterior lobe with curious acinar development resembling thyroid acini. The acinar cells had

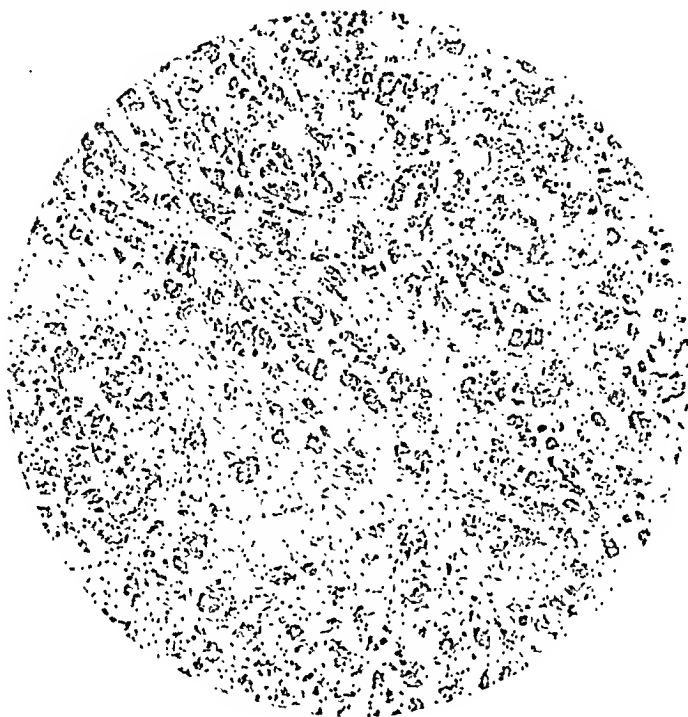


FIG. 8

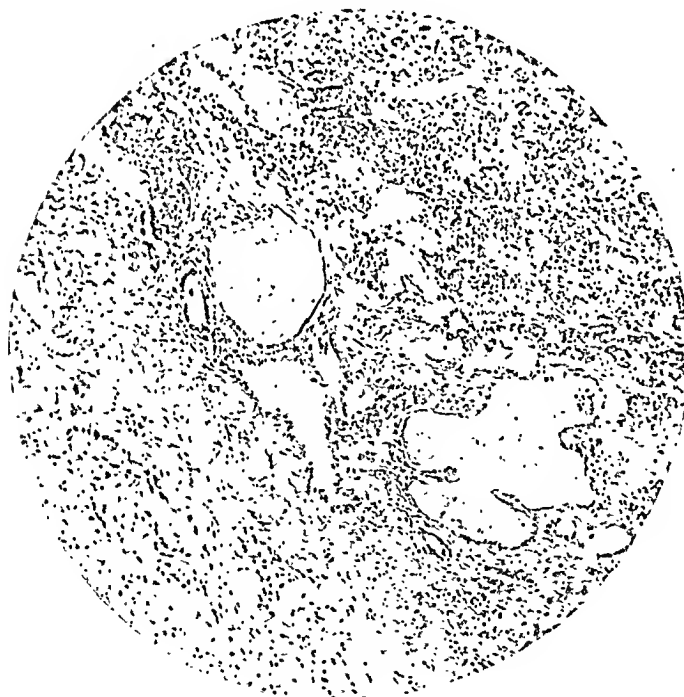


FIG. 9

FIG. 8. Photomicrograph of pituitary ( $\times 125$ ) showing diffuse fibrosis with acinar development resembling thyroid acini. Basophilic cells predominate.

FIG. 9. Section of pars intermedia ( $\times 125$ ).

a basophilic cast. The pars intermedia showed no notable morphological change. The pars nervosa appeared normal except for thickening of the capsule (Figures 8 and 9).

2. No evidence of adrenal tissue was found.

3. The thyroid showed extreme thyroid exhaustion, with marked lymphoid infiltration as well as degenerating fibrosis (Figure 10).

4. The lungs showed generalized emphysema and septal atrophy. They had the appearance of generalized mesodermal weakness. There was chronic pleuritis with low grade anthracosis, which were not clinically significant (Figure 11).

5. The kidneys showed slight indication of a presenile atrophy; otherwise there were no morphologic changes.

6. The pancreas showed no notable morphologic changes. There was plenty of insular tissue.

7. The liver showed stasis about the central veins, probably due to cardiac stasis. A small amount of infiltration with lymphocytes into Glisson's island was present. Otherwise, the liver appeared normal.

8. The perirenal fat showed atrophied fat tissue with collapsed cells, resembling that seen in aged persons.

9. The gall bladder showed postmortem autolysis.

10. A sympathetic ganglion was found in the perirenal fat which showed no notable morphologic changes.

11. The testes showed no notable morphologic changes except slight thickening of the tunica vaginalis (Figure 12).

12. The heart showed general atrophy but no fibrosis or sclerosis (Figure 13).

13. The brain showed no sclerosis of the vessels in the piaarachnoid, although there was a small petechial hemorrhage in this area (Figure 14).

14. The spleen showed moderate splenic fibrosis as well as a senile type of atrophy.

15. The diaphragm showed no notable morphologic change.

16. The aorta showed one moderately large patch of atheromatous sclerosis.

#### COMMENT

The case presents several interesting features which are subject to various interpretations. The clinical diagnosis of Addison's disease was substantiated by the autopsy, and as noted, not a trace of adrenal tissue was found.

How long would this patient have lived without therapy? We know that it is not unusual for patients to live this long and longer without much therapy, but the total absence of the adrenals, and the fact that negligence

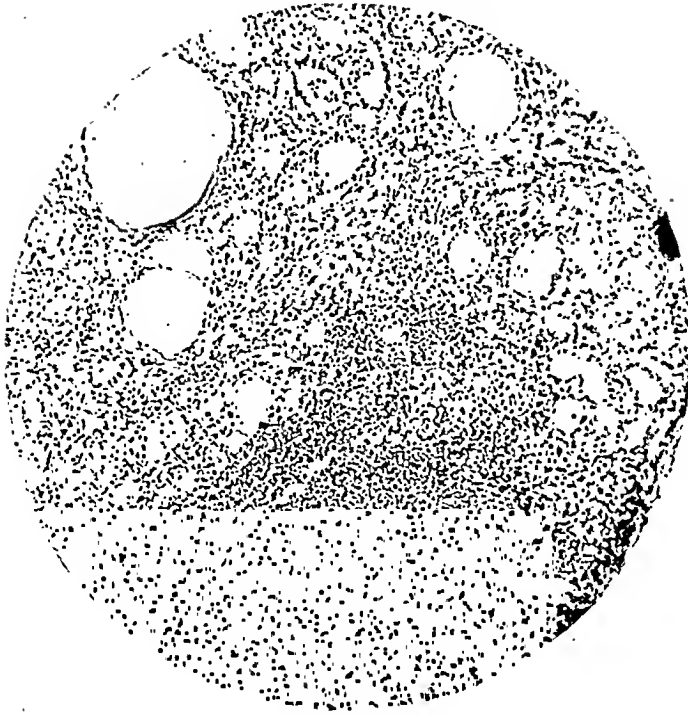


FIG. 10

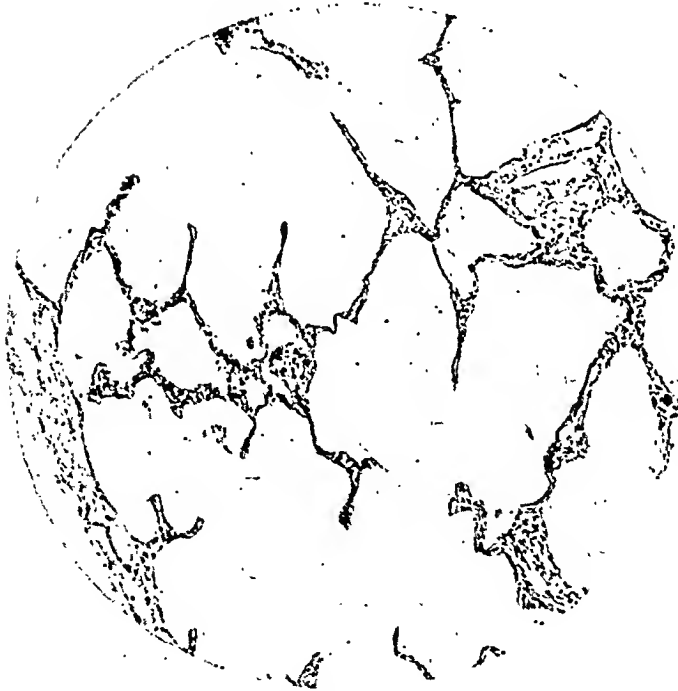


FIG. 11

FIG. 10. Section of thyroid ( $\times 125$ ) showing exhaustion, marked lymphoid infiltration and degenerating fibrosis.

FIG. 11. Emphysema of lung ( $\times 125$ ) with septal atrophy.



FIG. 12

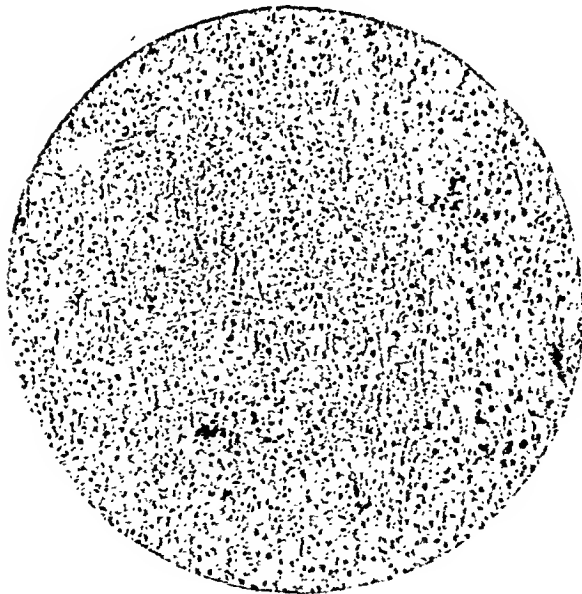


FIG. 13

FIG. 12. Section of testicle ( $\times 125$ ) showing normal structure.  
FIG. 13. Section of heart muscle ( $\times 125$ ) showing atrophy.

of therapy resulted in episodes of Addisonian crises and finally in death in one of these periods, lead to the supposition that the medication kept him alive. Such a severe involvement of the adrenals is, in itself, evidence that the therapy had prolonged the patient's life.

It seems justifiable to suppose that there was an anlage defect of the adrenals since a maternal aunt died of Addison's disease, the diagnosis having been verified by autopsy. Lending further support to this supposition is the fact that a niece of the patient (a brother's daughter) was showing pigmentary changes in the skin. It was this latter circumstance which as-

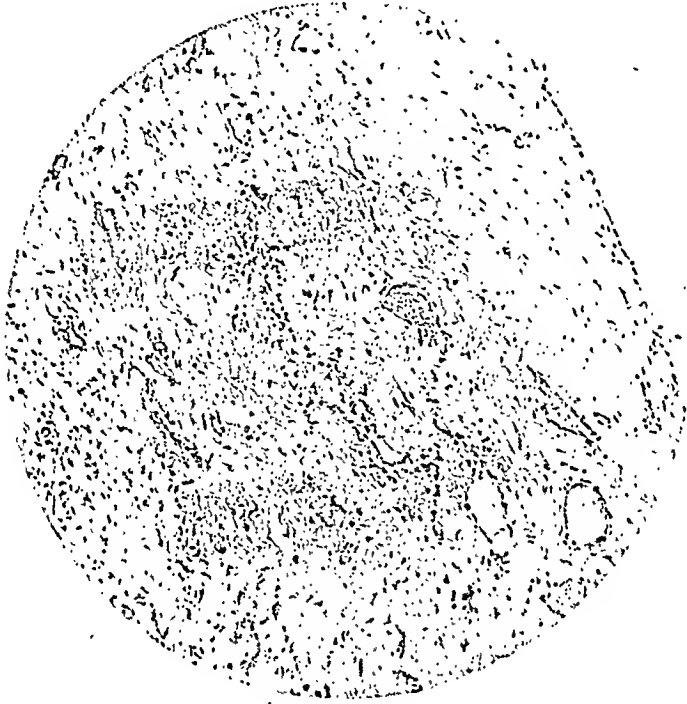


FIG. 14. Section showing hemorrhage in piaarachnoid ( $\times 125$ ).

sisted in obtaining the autopsy for we, and the family, were interested in knowing the effect desoxycorticosterone acetate therapy had on the tissues, particularly since a recent report by Forster and associates (1) had found some degenerative lesions in the cerebral arteries while other arteries studied failed to reveal any changes.

It was believed by these authors that the administration of the hormone, in moderate therapeutic doses over a long period of time, produced the cerebral vascular changes. Their case was that of a 13 year old boy, treated for two years. The patient herein reported received the drug for seven years without cerebral vascular changes, unless one includes the small hemorrhage in the piaarachnoid as such a vascular change. Sections of the

cerebrum, and other areas of the piaarachnoid, did not show vascular changes. Selye, Beland and Sylvester (5) described changes of the cerebral blood vessels in rats treated with large doses of the hormone, and salt.

The change in pigmentation is of great interest. The pigmentation anteriorly on the chest, neck and arms had already begun to fade within six months after beginning therapy with aqueous cortical hormone, salt and vitamin C. Rothman (4) said that control of the patient with cortical hormone and salt therapy is without appreciable effect on the pigmentation.

Attention has already been drawn to my oversight in the loss of pigment within the first six months. That there was a striking loss of pigment cannot be denied, as shown by the photographs, the colored movies, and the observations of my colleagues as well as the patient himself.

It is evident, however, that vitiligo or leukoderma is not an uncommon finding in Addison's disease, and whatever the mechanism is that produces the vitiligo may also be responsible for the loss of pigment.

Certainly no claim can be made that the therapy was responsible. I was of the opinion that the pigmentation of Addison's disease was due to the activity of the pars intermedia of the pituitary. The reason for this was given in an article by Bates and myself (3). The pars intermedia in this case showed no notable morphologic changes, and until such time as physiologic chemists can provide satisfactory clinical tests *in vivo* of this hormone, speculation is useless.

In the above mentioned article (3) we reviewed some of the literature relative to the state of the pituitary in Addison's disease, as well as after extirpation of the adrenals in animals.

Most autopsies of Addison's disease, in which the pituitary was studied, showed degenerative changes (3). There is evidence (3) that the pituitary attempts to compensate for adrenal cortex atrophy, but eventually undergoes atrophy.

The fibrosis of the whole anterior lobe with acinar development, resembling thyroid acini, is of interest. The cells were practically all basophilic.

The changes in the thyroid gland were those of an extreme exhaustion, with lymphoid infiltration as well as degenerating fibrosis. How much of this was due to pituitary stimulation, and to therapy, is, of course, unknown.

The lung changes of emphysema and septal atrophy seem to be part of the general exhaustion and weakness of tissues, and as expressed by the pathologist, Dr. Morse, "mesodermal weakness." No evidence of tuberculosis was found.



It is of interest that there was an abundance of pancreatic insular tissue. The low blood sugar found antemortem of 32 mg. certainly is a severe hypoglycemia, but no convulsions were present.

What of the pituitary-pancreatic-adrenal cortex relationship to the hypoglycemia, and the states of these glands during the life of the patient?

Was there sufficient pituitary activity to stimulate the pancreatic insular tissue and thus produce hypoglycemia, or was there insufficient contrain-sular hormone in the pituitary?

It is pure speculation, of course, whether the therapy with the 17-keto-steroids had anything to do with the formation of cholelithiasis, or whether the disease itself produced a change in cholesterol metabolism. Only one moderately large-sized patch of atheromatous sclerosis was found in the aorta.

The testes showed no morphologic changes of consequence, although they were grossly small. Potency had been restored following therapy, but in the later years he was reluctant to discuss his potency. He remained a bachelor.

#### SUMMARY

A case is reported of a 41-year-old male who had been followed for a period of nine years, and who had symptoms of Addison's disease for ten years. He had been treated with aqueous adrenal cortex extract, vitamin C, salt, implantation of 1,575 mg. of desoxycorticosterone acetate pellets, and intramuscular injections of this hormone, as well as by sublingual application. The latter method resulted in swelling of the salivary glands and salivation.

A change in pigmentation, previously unnoticed, occurred within six months after therapy with adrenal cortex extract and salt. Over a period of nine years, there was a marked loss of pigment. No claim is made that therapy was responsible for this loss of pigment.

Several episodes of Addisonian crisis occurred, requiring a stepping-up of treatment dosage. Failure to take treatment for two weeks resulted in an Addisonian crisis from which he did not recover. Exitus was preceded by delirium, fever, and hypoglycemia (blood sugar 32 mg.).

At autopsy, no adrenal tissue was found anywhere, and it was suggested that inasmuch as a maternal aunt had died of Addison's disease (verified at autopsy), and that a niece (a brother's daughter) was developing pigmentation, an adrenal anlage defect was postulated.

In addition to the absent adrenals, the important autopsy findings were:

1. The pituitary showed the following: diffuse fibrosis of anterior lobe, with curious acinar development, resembling thyroid acini; acinar cells

showing a basophilic cast; no changes in the pars intermedia; a thickened capsule of the pars nervosa.

2. The thyroid showed extreme exhaustion with degenerating fibrosis.
3. The lungs showed emphysema and a septal atrophy.
4. The pancreas had plenty of insular tissue.
5. Cholelithiasis was present.
6. The testes were small grossly, but normal morphologically.
7. The heart was small and showed general atrophy.

It is regretted that a section of pigmented skin which was removed at autopsy was lost.

#### ACKNOWLEDGMENT

The desoxycorticosterone acetate pellets were furnished through the courtesy of the Schering Corporation.

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# CO-EXISTING MYXEDEMA AND HYPERPARATHYROIDISM: CASE REPORT

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THE following case report is presented because in the first place it possesses the unusual feature of thyroid and parathyroid disease occurring in the same patient. In the second place it demonstrates many medical problems which emphasize the importance of considering hyperparathyroidism in syndromes with gastrointestinal symptoms. Finally, it suggests a psychosomatic problem: the relationship of alterations in calcium and phosphorus metabolism to the development of a major psychosis.

## CASE REPORT

The patient was a white female who came under observation for the first time in 1936 at the age of 50. Her complaints at that time were shortness of breath and palpitation of ten years' duration. There had been no recent increase in the severity of her symptoms but she was known to have had cardiac enlargement for ten years and had been previously regarded as suffering from arteriosclerotic heart disease.

Her past history was a full one. At the age of seven she had malaria. She was treated for tuberculosis in adolescence and for a possible recurrence at the age of twenty-five because of night sweats and chest pain. A tonsillectomy had been performed in childhood. A hemorrhoidectomy was done in 1913. There had been a salpingo-oophorectomy in 1918. A lipoma of the breast was removed in 1924 and a pelvic repair with amputation of the cervix was performed in 1936. Menopausal symptoms began at the age of 35 but menstruation continued until the age of 40. She had several miscarriages, her first and third pregnancies ending spontaneously at 3½ months.

On questioning, a history was elicited of loss of hair of several years' duration, drowsiness, and occasional swelling of the ankles. She also stated that she was extremely sensitive to cold.

Physical examination in June 1936 showed the hair of the scalp to be sparse but not especially coarse. The axillary hair was also sparse, but there was no loss of the outer third of the eyebrows. The pupils reacted briskly to light and accommodation. The neck was normal. The lungs were clear. The heart sounds were normal, there were no murmurs, and the blood pressure was 128/76. There was a slight scoliosis. The left knee was swollen, and there were bilateral bunions. The ankles were slightly edematous. Roentgenoscopy and a roentgenogram of the chest showed slight generalized cardiac enlargement. The hemoglobin was 75 per cent (Sahli). The electrocardiogram was within normal limits. Urinalysis showed a specific gravity of 1.012, no albumen or reducing substance and a normal sediment.

The diagnosis of myxedema was considered because of the presence of long-standing, non-progressive dyspnea, sensitivity to cold, loss of hair, drowsiness, cardiac enlargement

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and anemia. The basal metabolic rate was determined and was found to be minus 35 per cent. The patient was started on thyroid medication. She proved extremely sensitive to the drug, developing severe headache and palpitation, as is usual in myxedema, so that the initial doses had to be small. The dosage was increased gradually from 15 to 130 milligrams daily, with prompt improvement in her symptoms. In the spring of 1937, her bunions were operated on with a good result. In October 1937, she had an attack of

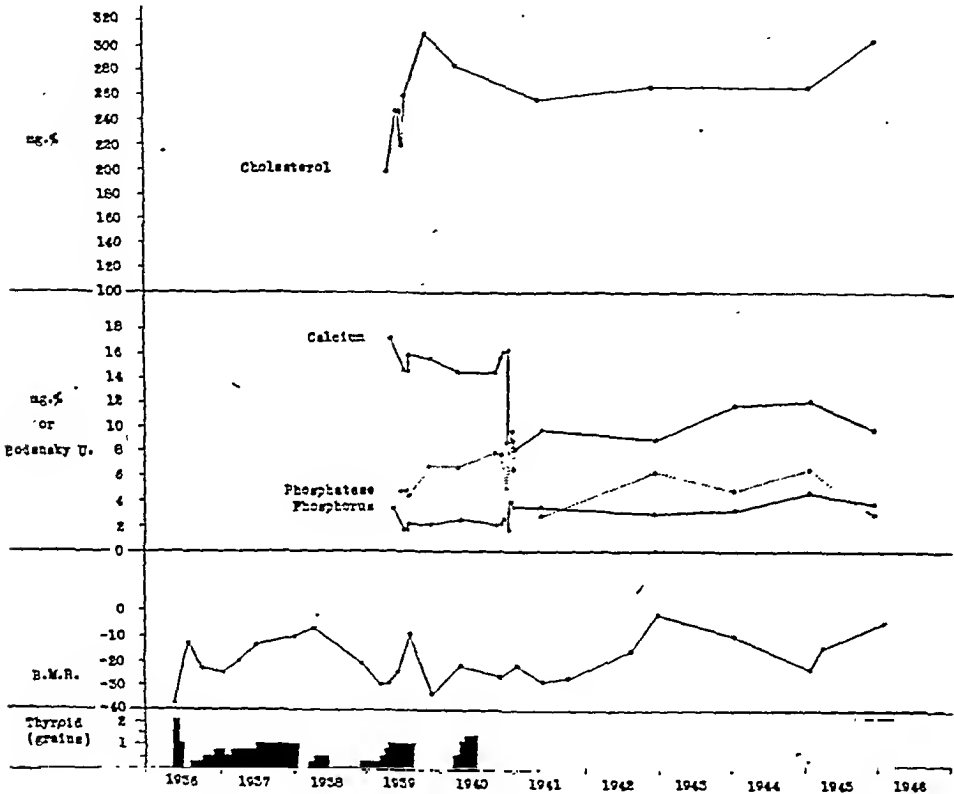


FIG. 1. Composite chart of the pertinent biochemical findings, the basal metabolic rates, and thyroid medication for the entire period of observation.

sciatica, but otherwise felt well. Her hair had stopped coming out, palpitation on effort was less, and her hemoglobin rose to 90 per cent. She was able to withstand cold without discomfort, and was no longer drowsy. Swelling of the ankles disappeared, and a roentgenogram of the chest showed a small but definite decrease in the size of the heart. The dyspnea, however, did not improve.

In December 1937, she developed a rectal fissure which required surgical treatment. In January 1938, she had an attack of "indigestion," with abdominal pain, vomiting, and passage of mucus by rectum. She was seen by a gastroenterologist who made a diagnosis of mucous colitis. During the summer of 1938, she developed chills and fever, pain in the left loin, and pyuria. An x-ray film showed the left kidney to be the site of many

calculi. Operation was advised but was delayed until January 1939, at which time a left nephrectomy was done for calculous pyonephrosis.

For a short time after the operation she felt better, but then began to complain of increasing constipation, frequent nausea and vomiting, aches and pains in various parts of the body, and weakness. Physical examination revealed no obvious cause for her complaints. There was no evidence of peripheral neuritis or arthritis, no anemia, and her BMR was minus 10 per cent. Her new complaints were clearly not due to insufficient thyroid therapy. To explain the more recent symptoms, the diagnosis of hyperparathyroidism was considered. This would explain the renal calculi, the generalized aches and pains, the weakness, and the nausea, vomiting and constipation. Accordingly, laboratory studies were made (by Drs. Henry L. Jaffe and Aaron Bodansky of the Hospital for Joint Diseases) and revealed the following information:

	Units	5/12/39	7/6/39	7/10/39	7/27/39
Calcium	mg%	17.0	14.5	14.4	15.7
Phosphorus	mg%	3.3	1.9	1.8	1.9
Phosphatase (alk)	Bodansky		4.5	4.5	4.3
Total Protein	Gm. %		5.9	5.9	6.0
Albumen	Gm. %		4.1	4.3	4.4
Globulin	Gm. %		1.8	1.6	1.6
A/G Ratio			2.3	2.7	2.7
N.P.N.	mg%		31.0	28.0	39.4
Cholesterol	mg%	196.0	245.0	219.0	257.0
Cholesterol Esters	mg%		177.0	157.0	184.0

Urinary excretion over a three-day period while on a calcium-poor diet was 611 mg., (200 milligrams or more is abnormal (4)). Skull x-rays showed minimal osteoporosis, and the long bones showed coarse trabeculation. A Sulkowitch test (3) of the urine showed marked excretion of calcium. A Hamilton test (11, 12) with a specimen of the patient's blood was done by Dr. Emil J. Bauman of Montefiore Hospital. This test is said to be a measure of circulating parathyroid hormone. The blood is injected into a rabbit while calcium chloride is given to the animal by stomach tube, and blood calcium determinations are made on the animal's ear blood. The test was, however, negative.

An electrocardiogram taken in 1933 showed a short QT interval. The actual figure was 0.32 second, the R-R interval 0.99. According to Bazett's formula (5),  $\text{systole} = K\sqrt{\text{cycle}}$ , in which K in women is  $.40 \pm .04$ , the QT interval should have been 0.36 to 0.44 second. Or expressed in the way that Barker, Johnston and Wilson (2) have suggested, K was .32, whereas it should have been  $.40 \pm .04$ . According to Ashman's formula (1)  $QT = K \log [10 (C + k)]$  in which C = cycle length, K = .385 to .390, and  $k = 0.07$ , the figure should have been .484 to .49. Other electrocardiograms taken in June 1936 and in April 1940 showed similar shortening of the QT interval so often seen in cases of hypercalcemia (14).

With all these evidences of hyperparathyroidism, an exploratory operation on the neck was performed on November 9, 1939, but no tumor of the parathyroids was found. Mediastinal exploration was advised but the patient refused. In the ensuing year, the persistence of the symptoms and blood chemistry findings indicated that she still had hyperparathyroidism, and on November 19, 1940 the patient was seen by Dr. Fuller

Albright at the Massachusetts General Hospital. The diagnosis was confirmed, and four days later a re-exploration of the neck was carried out by Dr. Oliver Cope but again without revealing any tumor of the parathyroids. A small papillary cystadenoma of the thyroid gland was removed, along with a specimen of the thyroid gland. The thyroid itself was normal on gross and microscopic examination. The adenoma was reported as "long-standing adenomatous goiter with calcification and secondary papillomatous

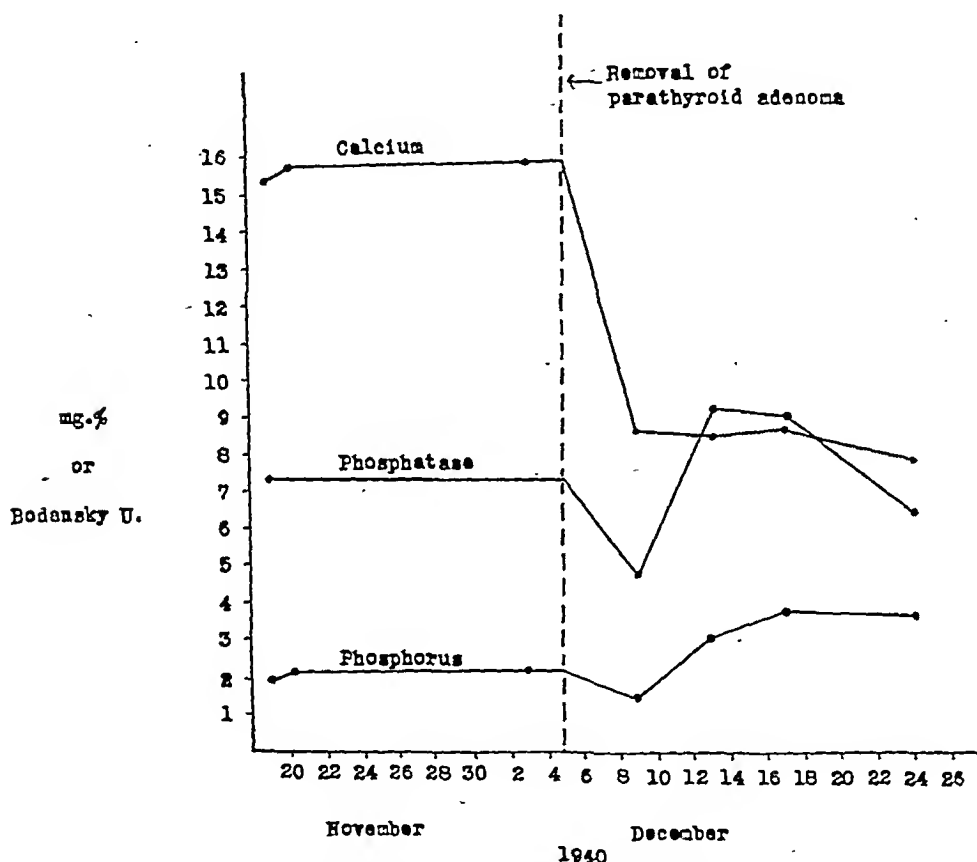


FIG. 2. Studies of blood calcium, phosphorus and alkaline phosphatase in the periods immediately before and after removal of the parathyroid adenoma.

change." On December 5, 1940, a sternum-splitting operation was performed; a parathyroid adenoma was located in the upper portion of the thymus gland and removed. Blood studies during the preoperative and postoperative periods are presented graphically in the accompanying charts. The pathologist's report was of a "tumor  $1.8 \times 8 \times 0.5$  cm. weighing 0.4 Gm. Parathyroid adenoma, glandular and cystic type." (8)

The postoperative course was marked by an incomplete atelectasis of the lower lobe of the left lung, and by a mediastinitis. There were symptoms of mild tetany, manifested by numbness and tingling of the fingers and feet, and a positive Chvostek sign, but these

disappeared by the seventh day. The rest of the course was uneventful, and the patient left the hospital on the twenty-third day after the second operation.

Subsequent follow-up revealed that, whereas the symptoms of hyperparathyroidism had disappeared, the picture of hypothyroidism remained unchanged. The patient had remained on a maintenance dose of 130 milligrams of thyroid daily, and the symptoms of myxedema had been controlled satisfactorily. An electrocardiogram on May 24, 1946 showed an increase in the QT constant of Bazett (5) to .41 (normal  $.40 \pm .04$ ), which is in keeping with the drop in blood calcium (8).

Following removal of the parathyroid adenoma, the patient was well for a few months, and then entered a period of hyperactivity followed by mental and emotional depression. At the same time, renewed menopausal symptoms became manifest, in particular frequent flushes, and vaginal smear showed the findings of estrogen deficiency. There was improvement in the flushes on parenteral estrogen therapy, but no striking change in mood ensued. In June 1941, six months after operation, she was depressed, cried very frequently, contemplated suicide, spoke of getting a divorce, thought that "life isn't worth living," and had "no interest in anything." This period of depression lasted about two to three months and then disappeared, only to recur, following a period of hyperactivity, in 1942. She also presented many symptoms suggestive of a peptic ulcer, but gastrointestinal x-ray studies at that time were negative except for a diverticulum of the sigmoid. She still received estrogenic hormone therapy for menopausal symptoms, but with no apparent effect on the depressed mood.

In 1945 she again had a period of hyperactivity followed by depression, with numerous gastrointestinal complaints unrelieved by estrogenic hormone therapy. In December 1945 she was again seen by Dr. Albright and then by Dr. Stanley Cobb who felt that these episodes of manic-depressive psychosis were possibly associated with the alteration in her estrogen and calcium and phosphorus metabolism. The serum calcium, phosphorus and phosphatase were, however, normal. Estrogen was resumed briefly and vitamin D, 50,000 units daily, was given for two months but discontinued when the patient felt and acted well. At present, with no therapy but thyroid for the last six months, the patient is asymptomatic.

### DISCUSSION

Hypothyroidism and hyperparathyroidism occurring in the same individual is no doubt coincidental. Hypothyroidism was manifested in this individual by shortness of breath, drowsiness, extreme sensitivity to cold, loss of hair, swelling of the ankles, cardiac enlargement, anemia refractory to iron therapy, an elevated serum cholesterol, and a low basal metabolism, all improving after thyroid medication. Hyperparathyroidism, while probably present even in 1933, judging by the short QT interval in the electrocardiogram, could hardly account for all the symptoms and signs. In fact, measurement of the basal metabolic rate in several cases of hyperparathyroidism was normal (9). Nevertheless it must be noted, in spite of such manifest symptoms of hypothyroidism, that except for the small papillary cystadenoma with calcification, the thyroid gland of this individual was normal both grossly and microscopically. One may merely speculate that the pituitary thyrotropic hormone in this patient was decreased.

The vomiting and constipation were the result of the hypercalcemia which existed prior to the removal of the parathyroid adenoma. That vomiting may be a prominent symptom in hypercalcemia should be emphasized. Recently, Rogers (15) reported two cases of hyperparathyroidism in which peptic ulcer was suspected but not found, and in which hyperparathyroidism was found only at necropsy.

Hyperparathyroidism should be suspected in all patients with calcium-containing kidney stones, polydipsia and polyuria, with generalized aches and pains, weakness, anorexia, nausea, vomiting and constipation. It is not always possible to examine the blood for calcium, phosphorus and phosphatase, and to x-ray the bones of the body. We have been making use of the following simple procedure when hyperparathyroidism is suspected. The patient is placed on a low-calcium diet (4) for three days. At the evening meal of the third day and thereafter until the test is completed, fluids are restricted. On the morning of the fourth day, a urine specimen is collected, its specific gravity is determined, and a Sulkowitch test (3) performed. If kidney function is good and the test reveals no calcium, hyperparathyroidism may be ruled out. Specific gravity of the urine of 1.022 or higher serves to rule out renal insufficiency. Keating and Cook (13) have mentioned a similar procedure.

Sulkowitch's reagent contains 2.5 grams oxalic acid, 5 cc. glacial acetic acid made up to 150 cc. with distilled water. Equal amounts of reagent and urine are mixed. The calcium in the urine is precipitated almost immediately as calcium oxalate.

The development of a cyclic psychosis for the first time in a woman of 55 years is rare (7). The fact that the symptoms appeared for the first time shortly after removal of the parathyroid adenoma makes one suspect a causal relationship. Perhaps the fall from an elevated to a normal blood calcium was responsible. The report of Rogers (15) mentions that in his Case 1, a negro man of 36, the psychiatrist found antemortem (no operation was done) "an agitated depression." Captain Martel, whose case was the first of hyperparathyroidism diagnosed in this country, was known to have changed his behavior after removal of his adenoma. From a calm individual, he became an apprehensive and agitated one. This was attributed to the sudden drop in his blood calcium. Hypoparathyroidism may be associated with major psychosis (6). It is possible that further information about manic-depressive psychosis may be obtained from long-term studies of calcium absorption and excretion. Some variability in the serum calcium levels in patients with psychoneuroses and psychoses has been found (6) but no conclusive evidence has been presented as yet.



## SUMMARY

1. A case of hypothyroidism and hyperparathyroidism, occurring simultaneously in the same individual, is presented.

2. The importance of gastrointestinal symptoms, namely, anorexia, nausea, vomiting and constipation, in hyperparathyroidism is emphasized.

3. A simple urine test of calcium excretion with Sulkowitch's reagent, following a 3 day low calcium diet and a half-day of restricted fluids, is suggested as a method of ruling out the presence of hyperparathyroidism. A negative calcium excretion with good kidney function speaks against hyperparathyroidism.

4. A manic-depressive psychosis followed removal of the parathyroid adenoma. This might have been due to the sudden change in calcium and phosphorus metabolism.

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# THE HYPEREMIA AZT AND THE EVALUATION OF THE HYPEREMIA RAT UNIT OF CHORIONIC GONADOTROPIN\*

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## INTRODUCTION

IN an earlier communication (7) we reported the results of 300 rapid pregnancy tests. Infantile female rats, which respond in a few hours to the presence of prolan B (luteinizing hormone—LH) with anterior pituitary reaction (APR) II, served in these tests as the experiment animals. APR II in the rat takes the form of a marked hyperemia, and is essentially different in character from APR II in the mouse and rabbit where prolan B induces follicle hemorrhages (blood points) (3).

The hyperemia AZT has been used routinely in this laboratory in a total of 2500 examinations. The test has proved to be a very reliable one, and in actual practice has completely replaced mouse (original AZT) and rabbit (Friedman's modification of AZT) tests. The error of the twenty-four hour hyperemia test, when the technic is sufficiently known, does not exceed that of the older AZT methods, i.e., 0.5-1 per cent. The two and six hour hyperemia tests, on the other hand, showed in our rats error rates of 31 per cent and 8 per cent respectively.

In developing the hyperemia test it became desirable to determine the minimum dose of prolan B (LH) which elicits a hyperemia reaction. As we have reported previously (7), the hyperemia reaction is elicited by prolan B in the ovary of infantile rats only if prolan A is present. Urine which contains only prolan A does not elicit the hyperemia AZT. During the last three years six to twenty-four hour hyperemia tests on different urines were run routinely concurrently with examinations in which the ninety-six hour original AZT rat method was employed. Attempts were further made to employ the hyperemia test quantitatively, since the assay of the chorionic gonadotropin is of fundamental importance in diagnosis of fetal death, mole, and chorio-epithelioma. In the course of this work, the surprising observation was made that rat unit values as determined by the hyperemia and the ninety-six hour test respectively, are only identical if an interval of eight to eleven hours is allowed in the hyperemia test between the injection

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and the examination. When time intervals outside this range were employed, serious discrepancies between the titers shown by the two methods were recorded. It was found in fact that the fluctuation of the rat unit value in the hyperemia test is a function of the time allowed between the injection and the examination.

The following experiments show how time affects the hyperemia AZT, and define the relationship of different gonadotropin units to the hyperemia unit as a function of this time factor.

### TECHNIC

Chorionic gonadotropin was in the form of a purified pregnancy urine preparation<sup>1</sup> which assayed 100,000 IU per Gm. The preparation was diluted with glucose to facilitate weighing. The diluted powder contained 1 IU in 12 mg. Suitable amounts of hormone were weighed out and dissolved in distilled water directly before each experiment to yield a concentration of 1 IU per cc.

The experiments were performed on a total of 700 infantile female rats, groups of 50 animals serving for an assay at each of the following time points: 1, 2, 3, 4, 6, 8, 10, 11, 12, 18, 20 and 24 hours. Controls of the estrus and luteinizing unit value of the gonadotropin solutions were run concurrently on groups of 10 infantile female rats.

### DETERMINATION OF HYPEREMIA UNIT (HU)

A hyperemia unit (1 HU) is the minimum amount of gonadotropic hormone which elicits in the rat ovary, when given by single injection, a red color similar in tone to that of spleen, kidney, or liver. The hyperemia reaction is unequivocally demonstrable ten to twenty-four hours after the injection. Two to ten hours after the injection the ovary reaction is pink rather than red and can be definitely detected only by an experienced observer through reference to the swelling with which hyperemia is associated. The difficulty of making the reading at this time interval is evident from the work of Farris (1) who obtained paradoxical results using the two hour test. If the reaction is read at ten hours or later, the positive color reaction is unequivocally demonstrable where a dosage greater than the threshold dose has been given. We have gathered the impression that the hyperemia unit is less liable to variation than the estrus and luteinizing units. A hyperemia unit (1 HU) is therefore defined as the minimum amount of hormone which elicits a positive reaction in both ovaries of a rat in each animal of a group of 4. The superior accuracy of the hyperemia test over

<sup>1</sup> We are indebted to Dr. M. L. Tainter, Winthrop Chemical Co., Inc., New York, for a supply of Korotrin.

the estrus and luteinization tests is probably due to the fact that the former is based on a quantitative variation involving progressively varying degrees of redness, whereas the latter two tests are based on qualitative changes (follicle maturation, ovulation, luteinization) which conform to the "all or none" principle.

#### DETERMINATION OF ESTRUS UNIT (RU)

An estrus unit (RU) is the minimum amount of gonadotropic hormone which produces a positive vaginal smear reaction (keratinization) seventy-two to ninety-six hours after injection of a single dose into a 3-4 weeks old rat weighing 25-30 Gm. (3 & 6). Vaginal smears are made at intervals of seventy-two, eighty-four and ninety-six hours after the injection. The unit is defined as the minimum amount of hormone which elicits a positive reaction in 50 per cent of the injected animals.<sup>2</sup>

#### RESULTS

The results of our examinations may be stated briefly as follows. The hyperemia unit is a function of the length of time which is allowed to elapse between the injection of the hormone and the reading of the response (cf. table 1).

Comparison of the values of HU and RU in table 1 shows that 20 RU are necessary to evoke a positive hyperemia reaction in the ovary two hours after the injection. The effective dose is found to decrease as the time lapse between injection and reading is increased. It may be noted that when the time span is six hours 14 RU are still necessary, but that at eight hours 1 RU suffices. Before eight hours the ovarian reddening is less than maximal. After ten hours the characteristic red reaction, which can be recognized by the resemblance of the color of the ovary to that of spleen, kidney, or liver, is produced. At ten hours the effective hyperemia dose (HU) is actually smaller than 1 RU; at this time interval, 0.5 RU suffices to produce a

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<sup>2</sup> For purposes of comparison the following additional units are given. Separate checks of these units were not undertaken in connection with the present study. In view of the practical importance of these units, expression of our results in these terms by conversions based on known data has seemed to us to be desirable.

*MU (mouse unit)* (3). A mouse unit is the minimum amount of gonadotropic hormone which produces a positive vaginal smear reaction (keratinization) 72-96 hrs. after injection into a 3-4 weeks old mouse weighing 8-12 Gm. The gonadotropin dose is subdivided into 6 injections. Vaginal smears are made at intervals of 72, 84 and 96 hrs. from the first injection. The unit is defined as the minimum amount of hormone which elicits a positive reaction in 50% of the injected animals.

*IU. The International Unit* can be calculated on the basis of the activity shown in the same test conditions by a standard preparation.

definite hyperemia effect. At terms greater than ten hours, the reaction becomes increasingly less sensitive, the number of RU which is required to produce a positive response increasing between twelve and twenty-four hours from 2 RU to 5 RU.

In routine examinations the following relations between the different units of chorionic gonadotropin were established:

1 IU = 2.5 MU = 5 RU (dose subdivided into 6 injections)

1 IU = 1.5 MU = 3 RU (single injection)

The quantitative relation of the different units is liable to variation depending on the strain of the test animal. In Germany, for example, the

TABLE 1. VARIATION OF THE HYPEREMIA UNIT ACCORDING TO THE LENGTH OF THE TIME LAPSE BETWEEN THE INJECTION OF GONADOTROPIC HORMONE AND THE READING OF THE RESPONSE

The RU was determined by parallel experiments. The IU was computed from RU with the help of a suitable calculation factor (0.2)

Time	2 h	3 h	4 h	6 h	8 h	10 h	11 h	12 h	18 h	20 h	24 h
HU:	1	1	1	1	1	1	1	1	1	1	1
RU:	20	16	15	14	1	0.5	1	2	3	4	5
IU:	4	3.2	3	2.8	0.2	0.1	0.2	0.4	0.6	0.8	1

senior author found the following relation:  $1 \text{ RU} = 1/5 - \frac{1}{8} \text{ MU}$  (4, 5). It seems probable from a remark of Rakoff (2) that the hyperemia reaction, too, possesses, in different strains of rats, different degrees of sensitivity.

## DISCUSSION

The experiments show clearly that a time factor is of great importance in the use of the hyperemia test for the quantitative assay of gonadotropin. We are convinced, however, that the hyperemia test is suitable as a basis of quantitative assay provided that a time factor table, like that which is presented in this paper, is accessible to the investigator.

The experiments show that the hyperemia test has maximum sensitivity if read ten hours from the time of the injection. At this time only 0.5 estrus units suffice to produce a positive hyperemia response. Need for care in interpreting the ten hour test is an obvious consideration. A ten hour test is positive not only to 250–500 RU per liter, a dose which also elicits a positive response in the usual test method (injection of 4 or 2 cc. urine and examination after 24 hours); it is found positive even with one-tenth of

this amount, i.e., as little as 25-50 RU per liter. The conclusion may be drawn that in a ten hour test for pregnancy a proper dose for injection is 0.2 and 0.4 cc. rather than 2 and 4 cc. of urine. Otherwise diagnostic confusion may arise between cases of pregnancy and conditions in which small amounts of luteinizing hormone occur in the urine. A 10 hour test with reduced urine dosages will be of advantage in the following circumstances:

- 1) determinations on rarely encountered toxic urine specimens;
- 2) determinations on small samples of urine;
- 3) tests in which rapid diagnosis has a high importance, e.g., extra uterine pregnancy.

Use of a ten hour test for ordinary routine examinations does not appear to be desirable, since its high sensitivity would lead, in cases of slight increase of urinary gonadotropin such as may occur even in a non-pregnant woman, to mistaken diagnoses of pregnancy. A high degree of reliability is afforded by the sixteen to twenty-four hour test which is, after all, quite rapid enough to meet the ordinary requirements of hospital and private practice.

#### SUMMARY

1. Chorionic gonadotropin elicits an early ovary reaction, anterior pituitary reaction II (APRII: Hyperemia reaction) in the infantile female rat. The hyperemia reaction does not fluctuate if the reading is carried out at a fixed time after the injection of the hormone. Large fluctuations are observed in the sensitivity of the test if the time span is allowed to vary between two and twenty-four hours. The hyperemia reaction is suitable for use in quantitative assay of gonadotropin provided that due allowance is made for the influence of the time factor.

2. Data summarized in table 1 show the variation of the sensitivity of the hyperemia test as a function of time span between the injection and the reading. The time-sensitivity curve has a parabolic form. Maximum sensitivity is found at ten hours when as little as 0.5 rat units is sufficient to elicit a hyperemic response. At eight and eleven hours the hyperemia unit equals the rat unit. The hyperemia unit (HU) rises progressively when the time span is varied below or above eight hours. At two and at six hours the HU is equal to 20 and 14 RU respectively. At twenty-four hours 1 HU is equal to 5 RU. The twenty-four hour test is four times as sensitive as the two hour test. The smaller reliability of the two and six hour tests as compared to the twenty-four hour test is explained by this finding.

3. The hyperemia reaction of the ovary at eight or less hours is pink. At ten hours or more, the ovary reaction is a deep red, similar to the color of spleen, kidney, or liver.

4. Using the twenty-four hour test, an accuracy of 99 per cent and better in pregnancy diagnosis was obtained. On the other hand, using the two and six hour tests to diagnose pregnancy, error rates of 31 per cent and 8 per cent respectively were experienced.

5. The ten hour test which affords the maximum sensitivity is recommended in the following special circumstances:

- (a) examinations of toxic urine of which only small dosages can be injected into a test animal;
- (b) cases involving suspicion of extra uterine pregnancy when speed of determination is a paramount consideration.

Disadvantages of the ten hour test as a routine examination are its extreme sensitivity and inconvenient time arrangement. Hence, the most suitable method for routine use is the eighteen to twenty-four hour test.

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# TWENTY-NINTH ANNUAL MEETING OF THE ASSOCIATION

FOR THE STUDY OF

## INTERNAL SECRETIONS

FRIDAY AND SATURDAY, JUNE 5-6, 1947

*Haddon Hall, Atlantic City, New Jersey*

## GENERAL INFORMATION

*Headquarters:* Chalfonte-Haddon Hall, Atlantic City, New Jersey.

Rates are as follows:	Chalfonte	Haddon Hall
Single room with bath:	\$6, \$7, \$9	\$7, \$8, \$10
Double room with bath (without ocean view):	\$8 and \$10	\$10 and \$12
Double room with bath (side ocean view):	\$12	\$14
Double room with bath (ocean front)	\$14 and \$16	\$16 and \$18

*Registration:* Everyone attending the meetings is requested to register. A fee of \$1.00 will be charged non-members of the Association. Membership cards should be presented when registering.

*The Scientific Sessions:* The Scientific Sessions will be held in the Viking Room of the Haddon Hall Hotel and programs will begin promptly on schedule. Papers presented at all meetings are planned for ten minutes and owing to the heavy schedule must be kept within this limit. Manuscripts of all papers should be submitted to the presiding officer or Secretary-Treasurer at the end of the presentation.

*Annual Dinner:* The Annual Dinner of the Association will be held on Friday evening, June 6, at 7:30 o'clock in the Rutland Room of the Haddon Hall, preceded by cocktails at 6:30 o'clock in the West Room. Secure tickets at time of registration.

*Council Meeting:* There will be a meeting of the Council on Thursday afternoon, June 5th at 2:00 o'clock, and a luncheon meeting on Friday, June 6th.

*Business Meeting:* The Annual Business Meeting of the Association and Election of Officers will be held at 4:30 p.m. June 7th, in the Viking Room of the Haddon Hall.

*Local Arrangements:* Dr. Matthew Molitch, 705 Pacific Avenue, Atlantic City, New Jersey, is in charge of the local arrangements for the meetings.

*Secretary-Treasurer:* Henry H. Turner, 1200 North Walker Street, Oklahoma City 3, Okla.



## PROGRAM

FRIDAY, JUNE 6

8:30 A.M. REGISTRATION

### I. 9:30 A.M. *Viking Room.*

FULLER ALBRIGHT, presiding.

1. STUDIES IN CORPUS LUTEUM FUNCTION.  
J. S. L. BROWNE, J. S. HENRY, and E. H. VENNING.
2. PROGESTERONE THERAPY OF UTERINE FIBROMYOMATA.  
ALBERT SEGALOFF, JOHN C. WEED (*by invitation*) and WILLIAM PARSON.
3. DISTORTION OF THE SPIRAL ARTERY IN THE OVARY IN THE PRESENCE OF CORPUS HEMORRHAGICUM CYSTS AFTER ADMINISTRATION OF GONADOTROPHINS TO RABBITS.  
S. R. M. REYNOLDS.
4. GONADAL STIMULATION FOLLOWING THE ADMINISTRATION OF ANTIGONADOTROPHIC SERUM.  
HERBERT S. KUPPERMAN, R. K. MEYER, and J. C. FINERTY.
5. LIVER AND GONADAL CHANGES FOLLOWING THE ADMINISTRATION OF CARBON TETRACHLORIDE TO MALE RATS AND FEMALE GUINEA PIGS.  
B. KRICHESKY, S. J. GLASS, E. FURLONG, and M. FEINER.
6. CLINICAL EVALUATION OF DIENESTROL, A SYNTHETIC ESTROGEN.  
A. E. RAKOFF, K. E. PASCHIKIS, and A. CANTAROW.
7. DIETHYLSTILBESTROL DIPALMITATE IN AQUEOUS SUSPENSION.  
S. CHARLES FREED.
8. THE EFFECT OF DIETHYLSTILBESTROL UPON ALLOXAN DIABETES AS RELATED TO FOOD INTAKE IN THE RAT.  
DWIGHT J. INGLE.
9. THE FACTOR OF PREVIOUS TREATMENT IN EXPERIMENTAL MENSTRUATION.  
DORIS H. PHELPS.
10. EXPERIMENTAL ALTERATION OF THE HUMAN OVARIAN CYCLE BY ESTROGEN.  
W. E. BROWN, J. T. BRADBURY, and A. F. JENNINGS.

#### PAPERS TO BE READ BY TITLE

11. STUDIES ON THE VARIATIONS OF BLOOD GONADOTROPHINS AND VAGINAL SMEARS DURING PREGNANCY IN CORRELATION WITH THE FETAL SEX.  
H. E. NIEBURGS, H. S. KUPPERMAN, and R. B. GREENBLATT.
12. ORAL ESTROGEN THERAPY DURING MENOPAUSE.  
ABBIE D. SELEY, DEBORAH BAUMGOLD, and SAMUEL VERNICK.
13. CORRELATION OF BASAL BODY TEMPERATURE CURVES WITH ENDOMETRIAL BIOPSY.  
A. R. ABARBANEL.

### II. 2:00 P.M. *Viking Room.*

14. A RAPID METHOD FOR THE DETERMINATION OF URINARY "17-KETOSTEROIDS."  
I. J. DREKTER, S. PEARSON, and T. H. MCGAVACK.
15. FURTHER STUDIES ON THE METABOLISM OF THERAPEUTIC DOSES OF THE NATURAL ESTROGENS IN HUMAN SUBJECTS.  
B. F. STIMMEL and C. L. STEALY.
16. A SIMPLE QUANTITATIVE COLORIMETRIC TEST FOR ESTROGENS.  
HERMAN COHEN and R. W. BATES.

17. URINARY STEROID BALANCE IN VIRILISM AND HYPOGONADISM.  
W. T. SALTER, F. D. HUMM, M. J. OESTERLING and W. W. ENGSTROM.
18. THE METABOLIC PATHWAY OF ESTRIOL PRODUCTION IN THE ORGANISM.  
MAX N. HUFFMAN and ARTHUR GROLLMAN.
19. COLOR REACTIONS OF THE STEROIDS.  
HERBERT JAFFE, BABETTE SOLOMAN and ROBERT H. WILLIAMS.
20. CHEMICAL ASSAY OF URINE FOR ADRENOCORTICAL HORMONES IN ENDOCRINE AND NON-ENDOCRINE DISEASES.  
WILLIAM H. DAUGHADAY, HERBERT JAFFEE and ROBERT H. WILLIAMS.
21. DISAPPEARANCE OF DIABETES MELLITUS ASSOCIATED WITH ACROMEGALY FOLLOWING ACUTE MASTOIDITIS AND BASILAR MENINGITIS.  
T. P. ALMY and EPHRAIM SHORR.
22. TRUE HERMAPHRODITISM: REPORT OF A CASE WITH AN OVOTESTIS, AND ENDOCRINE STUDIES.  
J. C. WEED (*by invitation*), A. SEGALOFF, WM. WIENER (*by invitation*) and J. W. DOUGLAS (*by invitation*).
23. TESTOSTERONE IN A CASE OF POLYOSTOTIC FIBROUS DYSPLASIA.  
RITA S. FINKLER and GEORGE M. COHN.
24. BILATERAL ARRHENOBLASTOMA WITHOUT MASCULINIZATION, ADENOMA TESTICULAIRE OF PICK.  
MINNIE B. GOLDBERG and ALICE F. MAXWELL.

PAPERS TO BE READ BY TITLE

25. SOCIAL AND PSYCHOLOGICAL REALJUSTMENT OF A PSEUDOHERMAPHRODITE UNDER ENDOCRINE THERAPY.  
RITA S. FINKLER.
26. SOME OBSERVATIONS ON THE UTILIZATION OF A LIQUID CHROMATOGRAM TECHNIQUE IN THE COLORIMETRIC ESTIMATION OF URINARY PREGNANEDIOL.  
BENJAMIN F. STIMMEL.
27. THE ORAL USE OF CRUDE ADRENAL CORTEX IN THE STIMULATION OF GROWTH OF THE FACE, PARTICULARLY THE CONDYLE OF THE MANDIBLE.  
FRANCIS M. POTTENGER, JR.

ANNUAL DINNER

III. 7:30 P.M. *Rutland Room, Haddon Hall*

PRESENTATION OF CIBA AWARD FOR 1947.

PRESENTATION OF E. R. SQUIBBS & SONS AWARD FOR 1947.

PRESENTATION OF AYERST, MCKENNA & HARRISON FELLOWSHIP FOR 1947.

WARREN O. NELSON, *Chairman of the Committee on Awards for 1946-47.*

PRESIDENT'S ADDRESS: FULLER ALBRIGHT, MASSACHUSETTS GENERAL HOSPITAL, BOSTON, MASSACHUSETTS.

SATURDAY, JUNE 7

IV. 9:00 A.M. *Viking Room.*

28. CHEMICAL AND CYTOCHEMICAL STUDIES OF THE RAT'S ADRENAL CORTEX FOLLOWING THE ADMINISTRATION OF PITUITARY ADRENOCORTICOTROPHIC HORMONE (ACTH).

H. W. DEANE (*by invitation*) and G. E. BERGNER (*by invitation*).

29. CHANGES IN CIRCULATING LEUCOCYTES INDUCED BY PITUITARY ADRENOCORTICOTROPHIC HORMONE (ACTH) IN MAN.  
A. G. HILLS (*by invitation*), P. H. FORSHAM (*by invitation*) and CLEMENT A. FINCH (*by invitation*). (*Introduced by K. EMERSON, JR.*)
30. RESULTS OF ADMINISTRATION OF ANTERIOR PITUITARY ADRENOCORTICOTROPHIC HORMONE TO A HUMAN SUBJECT.  
H. L. MASON, M. H. POWER, E. H. RYNEARSON, L. T. CIARAMELLI, C. H. LI, and H. M. EVANS.
31. METABOLIC CHANGES FOLLOWING THE ADMINISTRATION OF PITUITARY ADRENOCORTICOTROPHIC HORMONE (ACTH) IN MAN.  
G. W. THORN, F. T. G. PRUNTY (*by invitation*) and P. H. FORSHAM (*by invitation*).
32. URINARY URIC ACID-CREATININE RATIO FOLLOWING ADMINISTRATION OF PITUITARY ADRENOCORTICOTROPHIC HORMONE (ACTH) AS A SIMPLE TEST FOR ADRENAL CORTICAL FUNCTION.  
PETER H. FORSHAM (*by invitation*), F. T. G. PRUNTY (*by invitation*) and G. W. THORN.
33. FURTHER STUDIES ON THE PROTECTIVE POWER OF ADRENAL PREPARATIONS AGAINST BACTERIAL TOXINS.  
LENA A. LEWIS and IRVINE H. PAGE.
34. EFFECT OF TESTOSTERONE UPON THE EXCRETION OF GLYCOGENIC CORTICOID  
E. H. VENNING and J. S. L. BROWNE.
35. HEPATO-RENAL FACTORS IN CIRCULATORY HOMEOSTASIS: XVII RELATION OF ADRENALS TO FORMATION OF A RENAL VASO-EXCITOR PRINCIPLE.  
B. W. ZWEIFACH, E. SHORR, S. BAEZ, and S. ROSENFELD.
36. THE ROLE OF THE ADRENAL CORTEX IN PROTEIN CATABOLISM FOLLOWING TRAUMA.  
C. G. TOBY and R. L. NOBLE (*Introduced by J. B. COLLIP*).
37. A NEW HORMONE OF THE ADRENAL CORTEX.  
F. A. HARTMAN, K. A. BROWNELL, and J. S. THATCHER.
38. STUDIES ON OBESITY.  
R. H. WILLIAMS, W. H. DAUGHADAY, W. F. ROGERS, JR., HERBERT JAFFEE, S. A. ASPER, JR., and B. TOWERY.

PAPERS TO BE READ BY TITLE

39. PELLET THERAPY WITH DESOXYCORTICOSTERONE ACETATE IN ADRENAL CORTICAL INSUFFICIENCY.  
G. F. KOEFF and (*by invitation*) R. KIBLER.
  40. RELATIONSHIP OF SEX AND THE SEX STEROIDS TO THE ADRENAL GLANDS OF HAMSTERS AND RATS.  
H. S. KUPPERMAN and R. B. GREENBLATT.
  41. CORRELATIONS OF BIOCHEMICAL AND HISTOLOGICAL CHANGES IN THE ADRENAL CORTEX IN VARIOUS TYPES OF DISEASE.  
W. F. ROGERS, JR., and R. H. WILLIAMS.
- V. 2:00 P.M. *Viking Room.*
42. THE USE OF HYPERTONIC SALINE INFUSIONS IN THE DIFFERENTIAL DIAGNOSIS OF DIABETES INSIPIDUS AND PSYCHOGENIC POLYDIPSIA.  
A. C. CARTER and JACOB ROBBINS (*introduced by EPHRAIM SHORR*).

43. EFFECT OF AQUEOUS TESTICULAR EXTRACTS ON GROWTH AND DEVELOPMENT OF SPONTANEOUS MAMMARY TUMORS IN THE AGING BITCH.  
F. X. GASSNER.
44. CONJUNCTIVAL AND CORNEAL LESIONS IN HYPERCALCEMIA.  
J. E. HOWARD and F. B. WALSH.
45. A SECONDARY SEXUAL CHARACTER THAT DEVELOPS IN AN ORGAN COMMON TO BOTH SEXES BUT APPEARS NORMALLY ONLY IN MEN. WITH A DISCUSSION OF THE RELATION OF THIS CHARACTER TO ENDOCRINE STIMULATION.  
JAMES B. HAMILTON.
46. THE USE OF METHYL TESTOSTERONE AND TESTOSTERONE PROPIONATE IN PREMATURE INFANTS.  
E. K. SHELTON and J. S. MARK.
47. EQUINE PITUITARY GONADOTROPHIN AND ANTIHORMONE FORMATION.  
J. H. LEATHEN and A. E. RAKOFF.
48. STUDIES IN CASES OF PITUITARY TUMORS.  
K. E. PASCHKIS, A. CANTAROW, and A. E. RAKOFF.
49. CARBOHYDRATE APPETITE OF NORMAL AND HYPERTHYROID RATS AS DETERMINED BY THE TASTE THRESHOLD METHOD.  
CURT P. RICHTER.
50. THE EFFECT OF HYPOTHYROIDISM ON MENSTRUATION.  
W. O. THOMPSON, P. K. THOMPSON, and E. M. JEPPESON.
51. TWELVE CASES OF METASTATIC THYROID CARCINOMA STUDIED WITH RADIOACTIVE IODINE.  
S. M. SEIDLIN, E. OSHRY, and A. A. YALOW.
52. GOITER ON AN IODINE-FREE DIET GROWN BY HYDROPONICS, AND EXCLUDING ANY GOITER NOXA.  
J. F. MCCLENDON and W. G. FOSTER.
53. MITOTIC ACTIVITY AND WOUND HEALING IN THE CORNEAL EPITHELIUM OF RATS TREATED WITH THIOURACIL.  
W. FLEISCHMAN and I. A. BRECKLER.

PAPERS TO BE READ BY TITLE

54. CLINICAL MANIFESTATIONS IN FORTY CASES OF MYXEDEMA.  
D. SCHWIMMER, M. VOGEL and T. H. MCGAVACK.
  55. THIOURACIL IN THE TREATMENT OF HYPERTHYROIDISM COMPLICATING PREGNANCY AND ITS EFFECTS ON THE FETUS.  
M. JAMES WHITELAW.
  56. THE PERMANENCY OF ALLOXAN DIABETES AND THE STRUCTURE OF THE PANCREATIC ISLETS FOLLOWING CERTAIN EXPERIMENTAL PROCEEDINGS.  
RALPH G. JANES.
- VI. 4:30 P.M. *Annual Business Meeting.*

# Announcement

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## COUNCIL ON PHARMACY AND CHEMISTRY OF THE AMERICAN MEDICAL ASSOCIATION

The American Medical Association has entrusted the Committee on Therapeutic Research of the Council on Pharmacy and Chemistry with a fund to be expended in the promotion of investigations that may have therapeutic interest. The Committee invites applications for grants in this general field, which is conceived very broadly.

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COUNCIL ON PHARMACY AND CHEMISTRY

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## THE CONSTITUTIONAL TYPE OF PRECOCIOUS PUBERTY.

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“CONSTITUTIONAL” is the term used by Novak (28) to describe a type of precocious puberty for which no adequate cause has yet been found. This does not mean that a pathologic condition may not exist but that all the known conditions associated with sexual precocity have been excluded in the cases in question. Such conditions are the following:

(a) Granulosa-cell tumor of which there have been fully sixteen instances of its association with precocious puberty, Novak (28); three of these are described by Novak (27). An interesting observation made by this author (28) is that menstruation in such individuals, being of the estrogen-induced type is always anovulatory, which is in contrast with that of the so-called “constitutional” type; in one of the children to be described later on, pregnanediol was being excreted, showing that ovulation had occurred.

(b) Sexual precocity of the cerebral type has been recorded in seventeen cases (Weinberger and Grant (37)) in all of which lesions of the hypothalamus and third ventricle were present; girls are rarely affected with this lesion, though cases have been recorded by Dott (8), Bing, et al., (2) and by Gross (13) (his case 7). Ford and Guild (11) describe precocious puberty in two girls, aged 9, following measles encephalomyelitis and in a boy, aged 11, following epidemic encephalitis. These authors give a useful review of precocity due to brain tumors and add 7 cases to be ascribed to hypothalamic lesions caused by inflammatory processes; furthermore it is well

recognized that in cases of hydrocephalus a degree of sexual precocity frequently exists. Pineal tumors have been held to cause precocious puberty in boys but the sum of evidence to date (31) not only shows that the pineal gland is not a secretory structure but that the tumors in this region produced their effects either by their nature, e.g., as embryonal tumors, teratomas or mixed tumors or by their pressure effects on the hypothalamus or its nervous connections.

(c) In cases of sexual precocity one must also consider the possibility of adrenal cortical involvement, chiefly suspect in the sexually precocious boy. In a girl, hyperfunction of the adrenal cortex is almost always accompanied by virilism: hirsutism, deep voice, boyish figure and clitoris enlargement, but seventeen cases are on record in which menstruation has either preceded or occurred simultaneously with symptoms of heterosexual precocity. Halban (16) cites seven of these cases in all of which adrenal tumors were verified by autopsy; these and the remaining ten cases are referred to in the following table:

TABLE I. THE ASSOCIATION OF MENSTRUATION WITH ADRENAL TUMORS IN CHILDREN

Author	Age of child	Remarks
Mathias	3 years	Repeated menstrual periods.
Eigen	9 years	Menstruated once.
Bulloch and Sequeira	11 years	Menstruation and marked mammary development.
Schmidt	?	Menstruated once.
Lesser (25)	3 years	Menstruation followed by mammary enlargement.
Cooke (5)	7 years	Menstruation act. 4-7 years.
Kayne et al. (20) (cited by Halban (16))	7 years	Menstruation before virilism.
Walters et al. (35) (Case 2)	8 years	
Lasné	9 years	
(Riche	3.9/12 years	Aberrant adrenal tumors attached to the ovary.
Gaudier	4 years	
(cited by Reilly et al. (30))		
Hoag (19)	10 years	Only case out of 22 with hypernephroma.
Knewitz (22)	2 years	Adenocarcinoma found at aet. 5 years.
Freedman (12)	6 months	Menstruation and hirsutism.
Kepler et al. (21)	19 months	Menstruation followed by virilism
Harvey (18)	2½ years	Regular menstruation for 9 months, due to large cellular sarcoma of left kidney and adrenal.
Walters and Kepler (34)	2 years	2 or 3 menstrual periods.

In the majority of these patients hirsutism was present (or developed) and there was enlargement of the clitoris. Three of those enumerated are of special interest because menstruation and breast enlargement *preceded* development of any symptoms of virilism:

(i) Kayne et al. (20): menstruation in a girl aged 7 years was followed by amenorrhoea and the adoption of the male habitus with enlargement of the clitoris.

(ii) Lesser (25) is cited by Danforth (7) as describing a similar case; menstruation followed breast development when the child was 2 years old and lasted till  $3\frac{1}{2}$  years, when it ceased and hairiness developed so that at 6 years the child had a definite moustache and beard. At least one of the ovaries was enlarged.

(iii) Kepler et al. (21): menstruation and breast enlargement occurred in an infant of 19 months, who later developed symptoms of virilism and from whom an adrenal cortical carcinoma weighing 45 grams was subsequently removed. This sequence of events is probably of very rare occurrence.

(d) The gonadal group is the largest, as, in 21 out of 25 cases of precocious menstruation reviewed by Elterich (9), tumors or cysts of the ovaries were found, at operation or autopsy, and in 13 of these cases the tumor was of the nature of a sarcoma. To Elterich's total may be added the case of Fischer (10) in which menstruation in a child of  $4\frac{1}{2}$  years was associated with a cystic mass in the ovary with luteinization of the theca. Prior to operation the child excreted 31 mg. of sodium pregnanediol glucuronide which is equal to the amount excreted by a 5 months' pregnant woman. Elterich (9) encountered only one instance of "natural" or "constitutional" precocity—a girl aged 4 years; Novak (28) reports 9 cases of his own, Werner (38) two and Craven (6) one. With the possible exception of one case described by Young (39) (chap. 12) the author is not aware of any instance or precocity of this type in *boys*; two such are described in this report. At 4 years of age Professor Young's patient was 10 in. taller and 27 lb. heavier than a normal boy of his age; his muscular development was remarkable and sexual development was equal to that of an adult. Intellectually he was retarded, being equal to a 3 year old boy. Epiphyseal development was similar to that of a boy 10 years old. No pathologic condition was found at laparotomy, though the left adrenal was slightly larger than the right. It was not so large as the adrenal at birth and no part of it was removed. At that time estimation of the excretion of 17-ketosteroids was not a routine procedure for measurement of adrenal cortical function so that the possibility of cortical hyperplasia being the underlying cause of the precocity is not excluded, though such cases are exceedingly rare in boys, in spite of their relatively frequent occurrence in girls. This boy grew 6 in.



in 3 years (slower than the normal rate) and only  $\frac{1}{2}$  in. during the fourth year. At 8 he was stronger than a youth of 16.

The accelerated skeletal development described in the foregoing case is a characteristic of precocious puberty. According to Elterich (9), St. Hilaire was the first to call attention to it, and Lenz (24) found that osseous union of the epiphyses and diaphyses, normally occurring at the end of the second decade, was already complete in a 6 year old girl and the skeleton equal to one of 18 years. The pelvis in many cases resembles that of a fully developed female. Though at first taller than normal for their age, such patients are finally below the average height and even dwarf-like in appearance, owing to an early cessation of growth caused by premature ossification of the epiphyseal cartilages. This macrogenitosomia is not confined to isosexual precocity and, indeed, is even more marked in cases of adrenal cortical hyperfunction (whether due to tumor or hyperplasia of the cortex) in which heterosexual precocity occurs in the female child. This acceleration of osseous development occurs also in cases in which no adrenal involvement can be demonstrated, e.g., in precocity due to brain tumors and to ovarian and testicular tumors. It seems likely that the condition is caused by an interaction of adrenal, gonad and pituitary linked up with the metabolic processes.

Reference has been made to the fact that girls with the "constitutional" type of sexual precocity ovulate, whereas those in whom the condition is attributable to granulosa-cell tumor do not. Novak (28) found indubitable evidence of ovulation in three of his patients in whom corpora lutea were found at laparotomy. Further evidence of ovulation is provided by the fact that pregnancy has occurred in several cases of this kind. Elterich (9) counted 30 below 12 years old, children being born to mothers aged 5 $\frac{1}{2}$  years, 6 years (2 cases) and 9 years (cited by Novak (28)). This fact, together with the report that no lesion manifested itself in the later life of the patient (a normal menopause at 52 years is reported in one of the cases) (24), is strong suggestive evidence that the condition is a premature development of a normal process.

(e) A fifth series of cases of sexual precocity is that described by McCune (26) in which the condition was associated with bone cysts and pigmented nevi (xanthomatosis?). McCune described one such case, a girl aged 9 years, and Dr. Albright added thereto five similar cases in which menstruation started at 1, 7, 3, 4 and 7 years respectively. It was suggested that the association of the condition with precocious puberty might be due to the disturbance in cholesterol metabolism which occurs in xanthomatosis, cholesterol being a lipid chemically not very different from estrogen. Might it not be, however, that these bone cysts were those associated with a Hand-Schüller syndrome and that pressure on the hypothalamus had

given rise to sexual precocity instead of inducing the exophthalmos and diabetes insipidus usually found in this condition?

These 5 groups comprise the generally accepted causes of sexual precocity other than that of the "constitutional" type, which, in spite of the absence of controlled data regarding the gonadotropin excretion, has generally been attributed to "hyperpituitarism." A brief review of hormone excretion reported in the various types of sexual precocity will show how these may be differentiated, and will illustrate how little positive information has hitherto existed in the case of "constitutional" precocity.

McClune (26), in reporting his case, remarks that in addition to estrogen, which varied from 5-95 rat units per litre of urine, gonadotropin "was present periodically." In only one of Novak's (28) cases was the gonadotropin excretion estimated; Case 1 excreted "small amounts" of gonadotropin, while the estrogen excretion varied from less than 3 and 4 rat units to 20-25 rat units per litre. In five cases Novak observed that one ovary was enlarged and in almost every case the portion removed for biopsy contained small cysts and a retrogressing corpus luteum just below the surface. Allen (1) in Chap. 23 remarks—"Female infants with precocious puberty reveal in assay tests an amount of follicular hormone closely approximating that found in post-puberal girls." Menstruation due to granulosa-cell tumor is associated with very high values of estrogen excretion; those collected from the literature by Palmer (29) ranged from 65 M.U./litre to 17,390 M.U./litre, and the author recently recovered in one case >1200 I.U./litre. It should be noted, however, that Gross (13) reported values for estrogen equal to 175-650 I.U./24 hours in a child aged 2 years in whom menstruation proved to be due to an "astrocytic hamartoma" in the hypothalamus. Kraus (23) found an increased prolactin excretion in 60 per cent of the patients with cerebral tumors. Teratomas and some testicular tumors are also associated with an exceedingly high gonadotropin output. Precocity (whether isosexual or heterosexual) due to tumor or hyperplasia of the adrenal cortex is associated with high 17-ketosteroids excretion—values ranging from 20 to 300 mg./24 hours—and sometimes also with the excretion of considerable amounts of pregnanediol (14). Only one instance of the excretion of the latter is recorded in a case of precocious menstruation due to a luteal cyst (10): a child aged 4½ years who menstruated every 3 months from the age of 3 years and who was excreting 31 mg. sodium pregnanediol glucuronide daily.

#### METHODS

The following were used for hormone analysis:—

17-ketosteroids: Patterson's method, described by Hain (14).

Gonadotropin: a modification of Scott's kaolin adsorption method, described by Hain (14).

Pregnanediol: Venning's method (33).

Estrogen (combined): hydrolysis extraction by method I of Callow et al., (4), fractionation by their Method II; biological assay as performed by Hain and Robson (15).

### DESCRIPTION OF CASES

**Case 1.** (Record P409—Dr. Clifford Kennedy's patient)—A girl, aged 4 years, was admitted to the hospital Sept. 1943. Vaginal bleeding (a few spots) started when she was only 3 months old, and occurred again in Nov. and Dec. 1939. Bleeding occurred twice in 1940, 5 times in 1942, on each occasion a trace for approximately 4 days. A month before entering the hospital there was some vaginal bleeding for 7 days. On examination the patient was very well developed; mammary tissue and external genitalia were developed in advance of her age and the hymen was ruptured. She was mentally alert, with the reasoning power of a child of 7 or 8 years; a wayward and difficult child, she took several days to settle down in the ward. Height 45", weight 43½ lb., B.P. 85/60, r.b.c. 4.5 mill., w.b.c. 10,900, Hb. 89%, urea N 10 mg. %, Tot. protein 6.58 gm.%. Ossification = 6-9 years, development = 17 years; sella turcica normal. Hormone findings per 24 hours:

	<i>17-keto-steroids</i>	<i>sod. pregndl. gluc.</i>	<i>estrogen</i>	<i>A. Z. test</i>	<i>gonadotropin</i>
1943 (Sept.)	2 mg.	a trace	(free) 200 I. U.	not done	not done
1944 (Oct.)	3 mg.	4 mg.	(combined) <4 I. U.	negative	not done
1945 (July)	1.4 mg.	not done	not done	not done	16 M.U.

The ketosteroid output was only slightly raised, being equal to that of a girl of 6 or 7 years; the excretion of 4 mg. sodium pregnanediol glucuronide on the 12th day of a menstrual cycle indicated some luteal activity. On account of the high output of estrogen in 1943 an ovarian tumor was suspected and Dr. Kennedy was asked to explore the abdomen. The left ovary was larger than the right and on removal was found to contain follicles in all stages of development, and 2 atretic follicles, but no evidence of granulosa cell tumor formation.

There was no further menstruation, after this operation, for a whole year. Menstruation, lasting about 4 days, occurred in Sept. and Oct. 1944 and twice in March 1945 which is the last period for which we have data of the case. After the operation the child improved immensely and was much more amenable. There was no regression in her breast development during the two years following.

The amount of gonadotropin excreted by this patient—16 M.U./24 hrs.—is as much as an adult woman excretes; children of even 7 and 8 years of age do not excrete more than 4 or 5 M.U./24 hrs. It is interesting that this amount was being excreted 4 months after the last menstruation. It would seem that the initial stimulus which had started the menstrual rhythm (and also, presumably, pituitary secretion) was sufficient to maintain secretion at an adult level. The absence of menstruation may have been due to pituitary secretion not rising to high enough values.

**Case 2.** (Record P478—Mr. Broster's patient)—A girl, aged 3 years 7 months entered the hospital in Feb. 1944. White vaginal discharge when 1 month old; breasts prominent almost from birth; menstruation started at 19 months and occurred every 5 weeks lasting 4 days. Progesterone, injected at 2 years old, stopped periods for 6

months. Pubic hair at 2 months; had 18 teeth by 18 months, anterior fontanelle closed at 1 year; walked at 7 months, talked at 8 months. In an intelligence test when just 2 years old she was graded as  $4\frac{1}{2}$  years. Weight at 2 years 42 pounds; at 3 years 7 months 49 pounds, height 3'6".

Hormone finding per 24 hours:

	<i>17-ketosteroids</i>	<i>pregnanediol</i>	<i>estrogen (combined)</i>	<i>A. Z. test (double amount)</i>
Jan. 27, 1944	2.8 mg. (5.6 mg./litre)	a mere trace	<4 I. U.	completely negative
Feb. 18, 1944	2.4 mg. (4.0 mg./litre)			

At operation on March 3, 1944, the left ovary consisted of a large blood cyst about  $1'' \times 2''$  with a small amount of normal ovarian tissue. The right ovary—about  $\frac{1}{2}'' \times 1''$  was enlarged and contained a small blood cyst. Both adrenals were enlarged and granular. The uterus was larger than normal for her age. Her epiphyseal age was 12 years, but her teeth age was less than 6 (incisors not erupted). A scanty menstruation occurred 4 days after the operation. No note of this patient's gonadotropin excretion is available as this analysis was not being performed in 1944.

Case 3. (Record P498—Dr. Weiner's patient)—A girl aged 9 years 7 months, was admitted to Leeds General Infirmary March 1944 with a history that she had begun to menstruate at 8 years, had had 4 periods in the past 12 months and 2 during March 1944. There was nocturnal and diurnal enuresis. Two sisters aged 13 and 5 years respectively were quite normal. Axillary and pubic hair was present and breasts were greatly enlarged. Striae also were visible and there was impaired sugar tolerance. B.P. 125/95 to 100/55; r.b.s. 4.5 mill.; generalized obesity (91 lb., abdomen 30"). Height  $51\frac{1}{2}''$ , chest 32", length of lower half of body  $26\frac{1}{2}''$ . At X-ray her carpal centres were equal to those of a child 12 years old.

Hormone findings per 24 hours:

	<i>17-ketosteroids</i>	<i>pregnanediol</i>	<i>estrogen (combined)</i>	<i>gonadotropin</i>
Apr. 1944	$\left\{ \begin{array}{l} 3.0 \text{ mg. (i.e.} \\ 8.5 \text{ mg./litre)} \\ 3.4 \text{ mg.} \end{array} \right.$	"pregnane derivative"	<5 I. U.	not done
Apr. 1945	$\left\{ \begin{array}{l} 3.4 \text{ mg. (i.e.} \\ 6.8 \text{ mg./litre)} \end{array} \right.$	not done	not done	25 M.U.

The ketosteroids output was not above the average for a child of 9–10 years of age. Talbot et al. (1940) give the average excretion at 7–12 years as 4.0 mg./24 hrs.; the author's figure for girls aged 8–9 years is 2–3 mg./24 hrs. or 3.5–6.6 mg./litre.

The recovery of a "pregnane derivative" instead of pregnanediol in the acetone fractions of Venning's method has been reported by the author (14) in a case of adrenal cortical carcinoma when the patient was dying and has been encountered by her in a few women with virilism, in mastitis and in gynecomastia in men. The only other child in whom it has been recovered was K195, a female aged 16 months who had an enlarged clitoris and pubic hair, and who excreted 6.25 mg. 17-ketosteroids per litre. In case 3 "pregnane derivative" was recovered on the sixth day after menstruation began.

The excretion of 25 M.U. gonadotropin/24 hours in a child  $10\frac{1}{2}$  years old is definitely abnormal and represents the output of a normal adult. Unfortunately the stage of the

menstrual cycle at the time was not known. The impaired sugar tolerance, striae and enuresis are features suggestive of possible hypothalamic disturbance requiring this case to be put in a different category from the others in this series.

**Case 4.** (Record K172—Dr. J. B. Donald's patient)—A girl, aged 11 years, was admitted to the hospital Feb. 1946 on account of marked breast development and a history of having menstruated 3 weeks previously. Novak (28) considers that 8 or 9 years should be taken as the limit of "precocity" and that maturation after these years is within the limits of normality. This figure would be considered much too low for this country, 11–12 years being probably the lowest normal, so that possibly in the case of K172 the beginning of sexual development was not so accelerated as to be beyond normal limits. Patient's weight was 67 lb., height 52", span 49".

Hormone findings per 24 hours:

<i>17-ketosteroids</i>	<i>pregnanediol</i>	<i>gonadotropin</i>	<i>estrogen</i>	<i>A. Z. test</i>
4.4 mg. (i.e. 3.5 mg./litre)	slight trace	12 M.U.	<10 I. U.	negative

The ketosteroids output was normal; gonadotropin output was probably slightly in excess of the average for a girl of 11 but no data are available at present; the low estrogen excretion and negative A. Z. test excluded granulosa-cell tumor and embryonic or brain tumors as the underlying cause of the condition.

**Case 5.** (Record K158—Dr. R. B. Magill's patient)—A girl, aged 6½ years, developed normally until the last 6 months during which there had been excessive body-growth, accompanied by marked enlargement of the breasts. There was scanty pubic hair. Menstruation occurred in Nov. 1945 and Jan. 1946, just prior to consulting Dr. Magill. Patient has a sister aged 18 in whom menarche occurred at 14 years. On examination the uterus was found equal to that of a girl of 17, but the ovaries were small; the clitoris was normal. X-ray revealed no abnormality in the region of the pituitary; age of hands = 8 years; weight 57 lb., height 45½", span 47½". (Photo: Fig. 1.)

Hormone findings per 24 hours.

	<i>17-ketosteroids</i>	<i>pregnanediol</i>	<i>gonadotropin</i>	<i>A. Z. test</i>
Jan. 16, 1946	4.1 mg. (14.1 mg./litre)			{ completely negative
Jan. 28, 1946	5.0 mg. (6.9 mg./litre)	none	8 M.U.	

The ketosteroids are slightly higher than average for her age; so also is the gonadotropin output (which was taken on the 20th day from the start of a menstrual period lasting 7 days). Estrogen excretion was not ascertained directly but an excess such as accompanies granulosa-cell tumor would have shown itself in the A. Z. test, which proved completely negative.

An interesting fact regarding this child's mother was elicited by Dr. Magill: after the birth of the first girl she had two miscarriages due to fibroids for which an operation was performed 1½ years before the birth of the patient. The possibility that an abnormally high estrogen secretion (which, presumably, gave rise to the fibroids) had affected the fetus ante-natally, suggests itself. The length of the interval that elapsed in this case before any symptoms became manifest is difficult to explain, and such an explanation would seem more feasible in those cases in which vaginal bleeding commenced during the first year. It is possible, however, that a centre is stimulated which requires a certain period to elapse for its full function. The matter is discussed again later.



The secretion of gonadotropin is definitely higher than is normal for this age in K294 and K317 and probably also in K312, and justifies placing these patients in the category of the sexually precocious of "constitutional" type. All cases of this type should be observed periodically.

Only two boys with sexual precocity of the "constitutional" type have been encountered:

**Case 6.** (Record P519—Dr. Vining's patient)—Boy, aged 5 years, was admitted to Leeds General Infirmary Sept. 1944: 10" above average height, testes and penis = 15–17 years; pubic hair present; B.P. 105/75. Testicular biopsy revealed no interstitial cell hyperplasia but seminiferous tubules were almost adult in appearance and spermatozoa heads were seen in several tubules.

Hormone findings per 24 hours:

<i>17-ketosteroids</i>	<i>pregnanediol</i>	<i>gonadotropin</i>	<i>estrogen</i>	<i>A. Z. test</i>
2.25 and 5.0 mg.	"pregnane derivative"	17 M.U.	<10 I. U.	negative (also with 5.0 cc. urine)

As in other cases reported in this study the 17-ketosteroids are only slightly above the average for the age of the patient. The figure given by Talbot et al. (32) for children of 4–7 years is 1.3 mg./24 hrs., but the author has records of normal girls at 5–6 years of age excreting 2.0 to 2.5 mg./24 hrs. Boys tend to excrete slightly higher values than girls of the same age.

The excretion of 17 M.U. gonadotropin/24 hrs. is on a par with the adult values obtained in the other members of this series of "precocious" children. Normal children of the same age excrete from <2 to 4 M.U./24 hrs.

**Case 7.** (Record K263—Dr. J. B. Donald's and Mr. G. L. Alexander's patient)—A boy aged 6½ years, height 4'5", weight 44 pounds, well-developed and proportionate; adult genitalia, axillary and pubic hair well-grown; mustache beginning. Voice broke at 4½ years, now baritone. Adolescent acne since 4½ years. Ossification = boy of 14 years; no skeletal deformity. Sella turcica not enlarged; no polydipsia or polyuria. Nothing abnormal in mother's or family's history. (Photo: Fig. 2.)

<i>17-ketosteroids</i>	<i>gonadotropin</i>	<i>A. Z. test</i>	<i>5.0 cc. urine per immature mouse</i>
6.0 mg.	18 M.U.	completely negative	no effect

Again the 17-ketosteroid excretion was slightly above normal; it was in keeping with his precociously mature condition, as was also the gonadotropin output, similar values having been obtained by the author in a normal boy of 14. The absence of any reaction in the A. Z. test and when 5.0 cc. of urine was injected, indicated that there was neither a testicular nor an embryonic tumor to account for the precocity.

## DISCUSSION

Hormonally all the members of this group of cases have in common an excretion of gonadotropin such as is found only after puberty; the values recovered were 16, 25, 12, 8, 22, 7, 13, 17, and 18 M.U./24 hrs. In addition the excretion of 17-ketosteroids was above the average for the age of the patient, but well below values associated with hyperplasia or hyperfunction

of the adrenal cortex, conditions which are associated with an excretion of 20-300 mg./24 hrs. It is likely that, in such patients, pituitary function as a whole is at an increased rate so that not only is gonadotropin secretion in excess of normal but also the secretion of corticotropic hormone; thus the adrenal cortex participates in the precocious maturation which the gonads display. In the two boys that have been included in the series, a

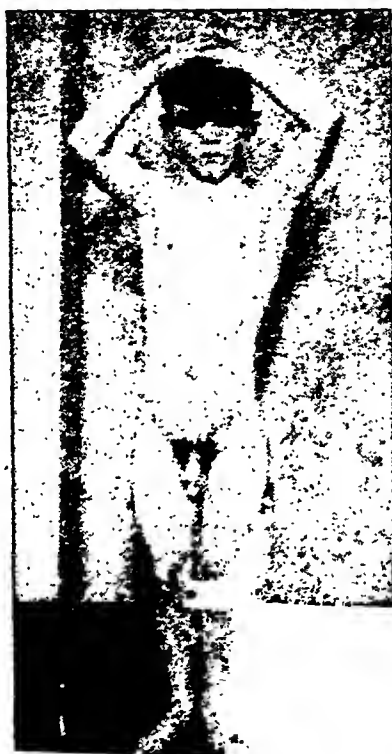


FIG. 2. Case 7—Dr. Donald's and Mr. Alexander's patient: age  $6\frac{1}{2}$  years.

little of the androgen may have come from testicular function as the gonads were mature in each case.

The absence of any history of illness which would be likely to affect the brain and ultimately the pituitary, by way of the hypothalamic centres, taken in conjunction with the only slightly raised excretion of gonadotropin, makes it highly improbable that there was any pituitary overactivity due to chronic increased intracranial pressure. The urine of such patients, according to Kraus (23), is so rich in follicle-stimulating hormone that only 3.0 cc. causes follicle-ripening when injected into immature mice. In every one of our cases this amount was completely ineffective.



The fact that in so many cases precocious menstruation has been followed by normal function throughout life and that there have been thirty instances of motherhood at 12 years of age and under (9) demonstrates that such an occurrence is not necessarily pathologic. It is of the greatest importance to differentiate, by hormone assays, between the pathologic and the merely "precocious" condition.

As to why an otherwise normal mechanism should be awakened at an abnormally early age, no satisfactory explanation seems forthcoming. Novak (28) assumes a chromosomal or genic basis as the most plausible, hence his term "constitutional." In one instance in our series—but alas! only one—a history of fibroids suggested the possibility of intrauterine action of estrogen upon the fetus. The delay in the manifestation of "precocity" has a counterpart in the delay in its occurrence after inflammatory processes in the brain, viz. 12, 7 and 8 years (11). It would seem that years elapse, after the initial stimulus is given to set en train the course of puberty, before outward signs become manifest.

Reference has been made to the recovery in two cases of a substance allied to pregnanediol (since it is obtained by the Venning method used for isolation of sodium pregnanediol glucuronide) which we have called a "pregnane derivative." Since the only occasion on which pregnanediol is excreted other than in pregnancy and the luteal phase of the menstrual cycle is in hyperplasia or tumor of the adrenal cortex, it seems likely that the "pregnane derivative" is the result of abnormal function of the adrenal cortex. It would be fallacious to argue that sexual precocity of the type herein described is due to abnormal adrenal function as it is quite possible that the latter is the result of the former. If, however, for purposes of argument, abnormal cortical function is taken for granted, its existence as the result of maternal hypersecretion of estrogen ante-natally is intelligible since estrogen does cause hypertrophy of the adrenal cortex (3). The suggestion need not be carried too far, as it is entirely a matter of speculation. The sequence of menstruation and adrenal cortical abnormality carries weight from the number of instances in which such abnormality has subsequently developed (see Table I.)

To describe cases of "constitutional" precocity as being due to "hyperpituitarism" is misleading. Such a term as "hyperpituitarism" is rightly applied to sexual precocity following on teratomas and cerebral tumors or inflammatory processes in the brain in which there has been destruction of structures which normally inhibit anterior pituitary activity through the hypothalamic channels. In such cases pituitary secretion is so excessive that either as little as 3.0 cc. of urine gives a positive A. Z. reaction (i.e. blood points in the ovaries of immature mice) or with 5.0 cc. of urine marked uterine enlargement is obtained, indicating the excretion of some

hundreds of Mouse Units of gonadotropin per 24 hours—a very abnormal condition. The cases that have been described in this report are examples in which a normal mechanism has been set in motion, but prematurely.

Novak (28) in reporting his cases makes certain wise recommendations: (a) to examine the patient at regular intervals, especially if no operation was performed; and (b) to stress the necessity for careful psychological management of such children, to avoid selfconsciousness in the child and a sense of inferiority or of abnormality, as well as to ensure protection against insemination.

#### SUMMARY

1. The various causes of sexual precocity are enumerated and the “constitutional” type is defined.

2. Five girls and two boys with sexual precocity of the “constitutional” type are described and their hormone output is given.

3. Excretion of gonadotropin and of 17-ketosteroids in excess of the normal for the age of the patients is common to all, the gonadotropin output being equal to that found in many adults.

4. In two cases a “pregnane derivative” was excreted instead of pregnanediol.

5. Possible reasons for the early awakening of a normal mechanism are discussed.

#### ACKNOWLEDGMENT

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# AN EVALUATION OF THE URETHRAL SMEAR AS AN INDEX OF ANDROGENIC DEFICIENCY IN THE MALE

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IN a communication to this Journal Andrews (1) suggested the use of urethral smears as an index of androgen deficiency in males. He reported that smears of the distal cells of the male urethra showed characteristic staining reactions with the Shorr stain (2) indicating the presence or absence of an androgen deficiency. The urethral cells of normal individuals were said to appear as masses of cuboidal cells with rather pink staining cytoplasm and with very distinct brown nuclei. On the other hand, where an androgen deficiency was present the cytoplasm stained blue and the nuclei red. Where androgen therapy was given to correct a deficiency of this hormone, the staining reaction of the urethral smear was found to change, eventually becoming characteristic of the normal individual. Such findings, if confirmed, would indeed have much clinical diagnostic significance. We have undertaken a study of this technic with results which do not support the observations reported by Andrews.

## METHODS

A urethral smear is taken by spreading open the meatus and applying a dry slide to the inner moist surface. The slide is fixed immediately in a solution of equal parts of ether and 95% alcohol and then stained with the Shorr stain. We have found that if more than one to two seconds elapse between the taking of the urethral smear and its fixation, a "dry smear" results in which most cells will take a diffuse orange-red stain. This distortion of the staining reaction by the drying of the smear before fixation can obviously lead to an erroneous interpretation of its cytology.

Shorr (2) has described the use of a mixture of equal parts of glycerin and 95% alcohol for the prevention of drying of the vaginal secretion before fixation in certain cases in which very scant vaginal secretion is present. We have used such a glycerin-alcohol mixture for preparing urethral smears in the present study by the following procedures: 1. A drop of the mixture is spread thinly over the slide and this area applied several times to the inner lips of the meatus. 2. A drop of the mixture is placed in the meatus by means of a glass rod before applying a dry slide to this region. 3. A glass rod moistened with the mixture is introduced about one-fourth of an inch

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within the meatus, the rod rotated three or four times, and the urethral cells thus obtained are spread on a slide. The smears obtained by these procedures are promptly fixed in the ether-alcohol solution and stained in the usual manner.

Fifteen normal and fifteen hypogonadal males were studied in this series.

### RESULTS

No significant differences were observed in the staining reactions of the urethral smears of normal and hypogonadal subjects. In both groups a mixed cytological picture was obtained, the smears consisting of typical cornified squamous cells and non-cornified cells of varying shapes and sizes. In the smear obtained by the direct application of the slide to the meatus according to the method of Andrews and fixed promptly, the non-cornified cells occupied the central area of the smear while the cornified cells were usually distributed in the periphery. This suggested that the non-cornified cells had their origin in the moist urethral mucosa, while the cornified cells were largely derived from the mucocutaneous junction of the glans and mucosa. This was shown to be the case by the use of the glass rod technic in which the rod was moistened with the glycerin-alcohol mixture and applied directly to the moist surface of the urethra. Smears thus obtained were virtually devoid of cornified cells.

Smears taken by any of the technics employed in this study consisted predominantly of non-cornified cells of a wide variety of shapes and sizes and with a cytoplasm which stained a bluish green. They included small cuboidal cells with large nuclei and small amounts of cytoplasm, somewhat larger round or oval cells with large nuclei containing chromatin granules, larger squamous cells with large vesiculated nuclei, and thin wafer-like squamous cells of large size containing small pyknotic nuclei. Except for the small cuboidal cells, and rarely, a few columnar cells, these non-cornified cells were counterparts of cells observed in the vaginal secretion. The cornified cells consisted of two types, a large flat squamous cell with a small pyknotic nucleus, and nondescript irregular cells frequently devoid of nuclei. The percentage of the various non-cornified cell types changed from day to day, and even on the same day in the same subject. These variations in cell types and the absence of detectable significant differences in the urethral smears of normal and hypogonadal males invalidate the diagnostic significance of the urethral smear as a measure of androgenic hormone formation.

In addition to the above study, two normal males were given 5 mg. of estrone sulfate (Premarin) daily for two weeks by mouth; following this, daily injections of 10,000 R.U. of estradiol benzoate were given to one subject for two weeks and to the other subject for three weeks without there being any significant changes in the urethral smear. In the latter sub-

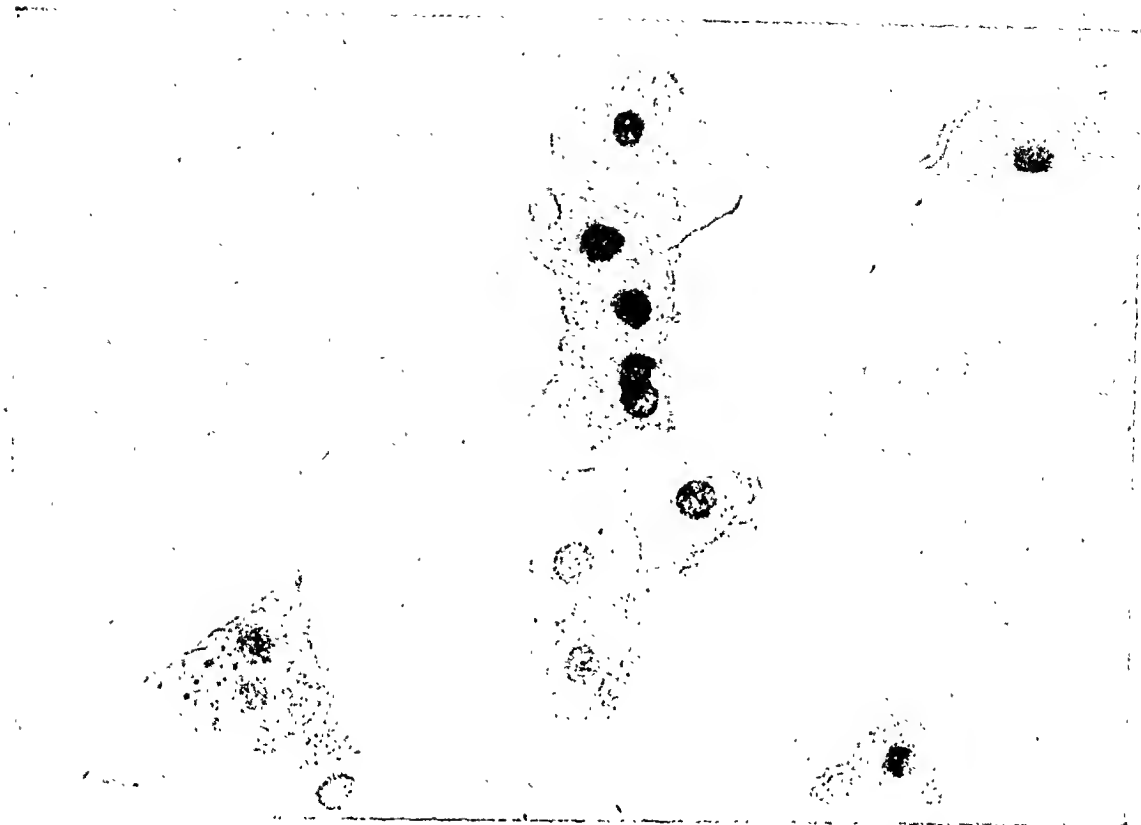


FIG. 1A. Urethral smear of a normal male C.E., age 34; urinary 17-ketosteroid excretion, 14.3 mg./24 hrs.  $\times 600$ .

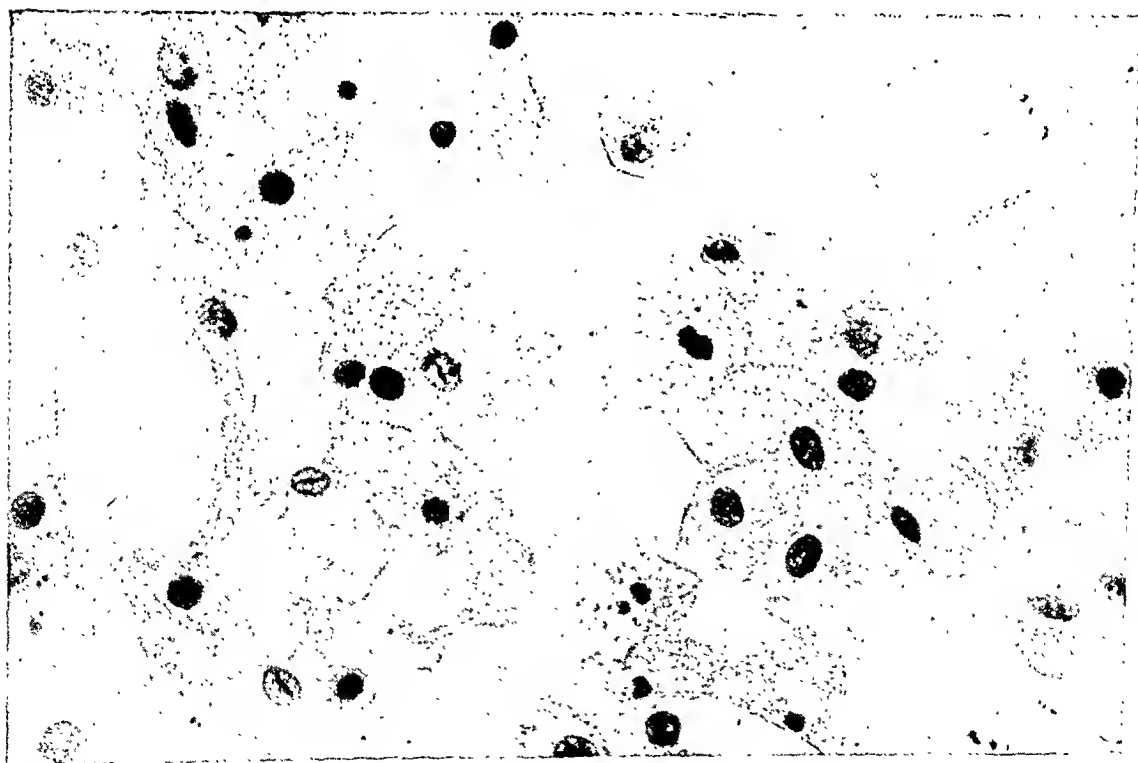


FIG. 1B. Urethral smear of the same subject C.E. after receiving 5 mg. of estrone sulfate (Premarin) daily for two weeks by mouth and then daily injections of 10,000 R.U. of  $\alpha$ -estradiol benzoate for three weeks.  $\times 600$ .

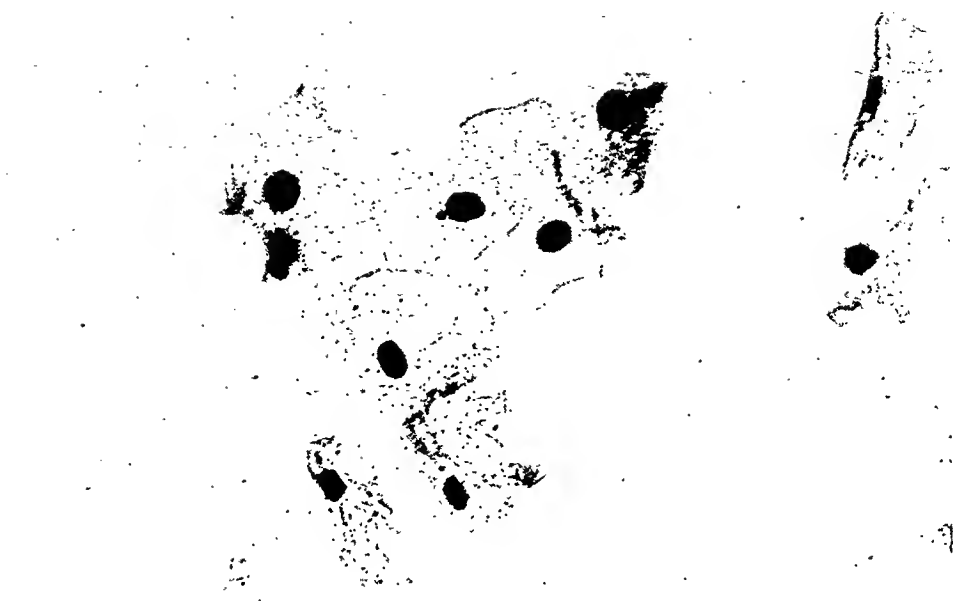


FIG. 2A. Urethral smear of a hypogonadal male W.S., age 35; urinary 17-ketosteroid excretion, 7.0 mg./24 hrs. (values for normal males in this laboratory, 9-16 mg./24 hrs.).  $\times 600$ .

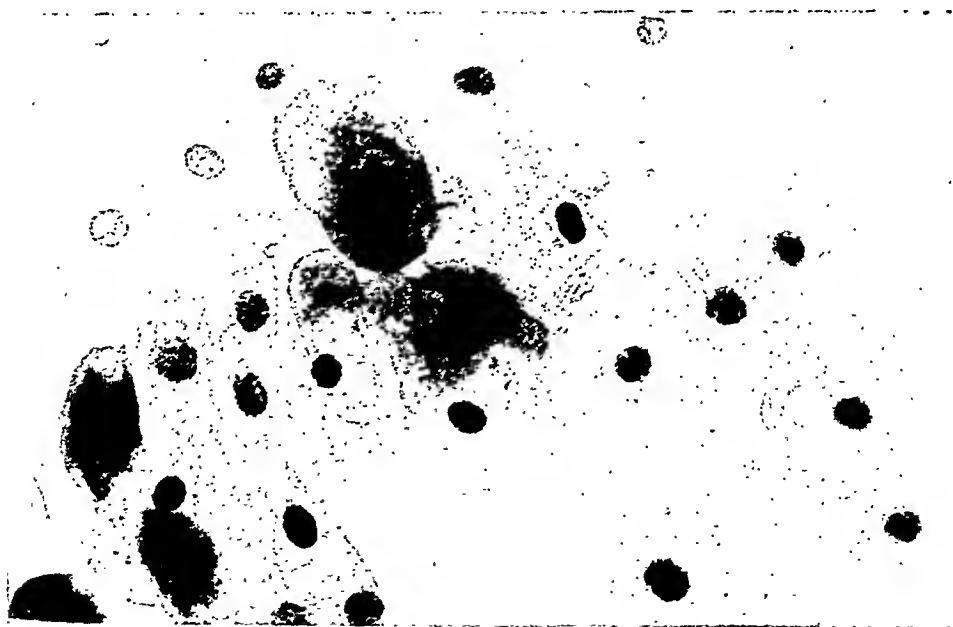


FIG. 2B. Urethral smear of the same subject W.S. after the oral administration of 20-30 mg. of methyl testosterone daily for 2½ months resulting in an increase in size of the penis, increase in libido, potency and hair growth, all indicating adequate replacement therapy.  $\times 600$ .



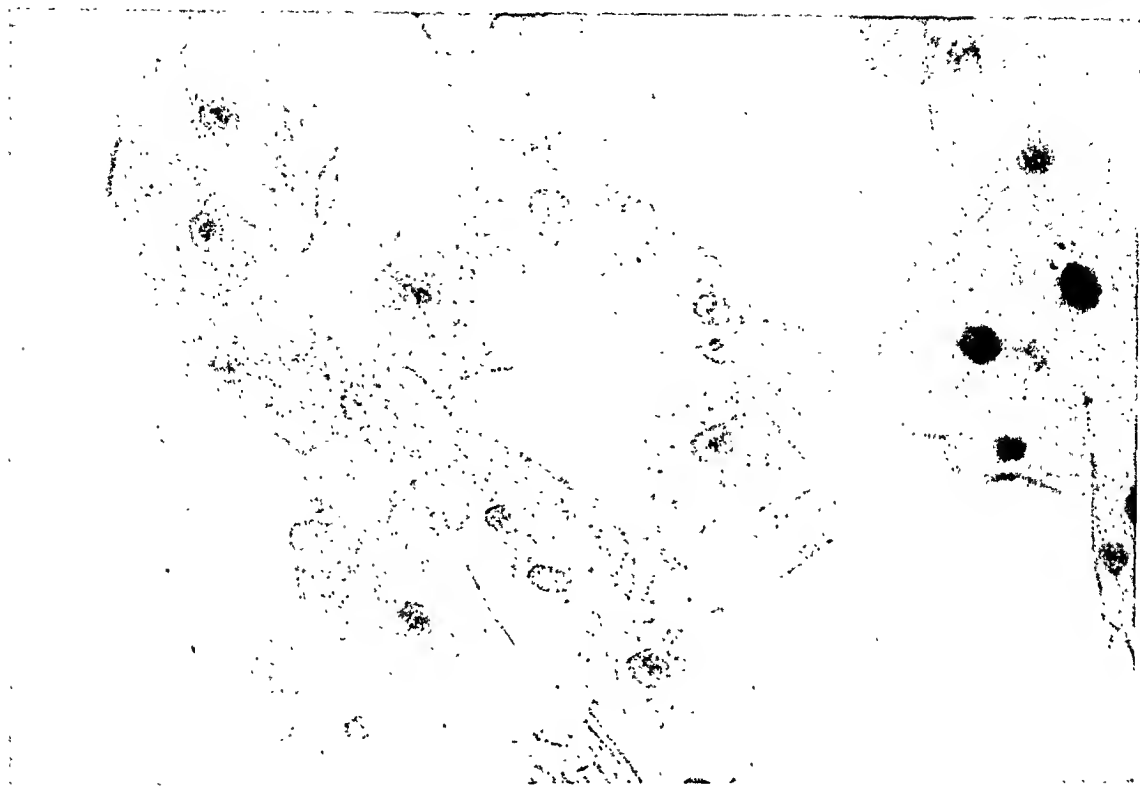


FIG. 3. Urethral smear of a hypogonadal male E.K., age 47; urinary 17-ketosteroid excretion, 3.1 mg./24 hrs.  $\times 600$ .

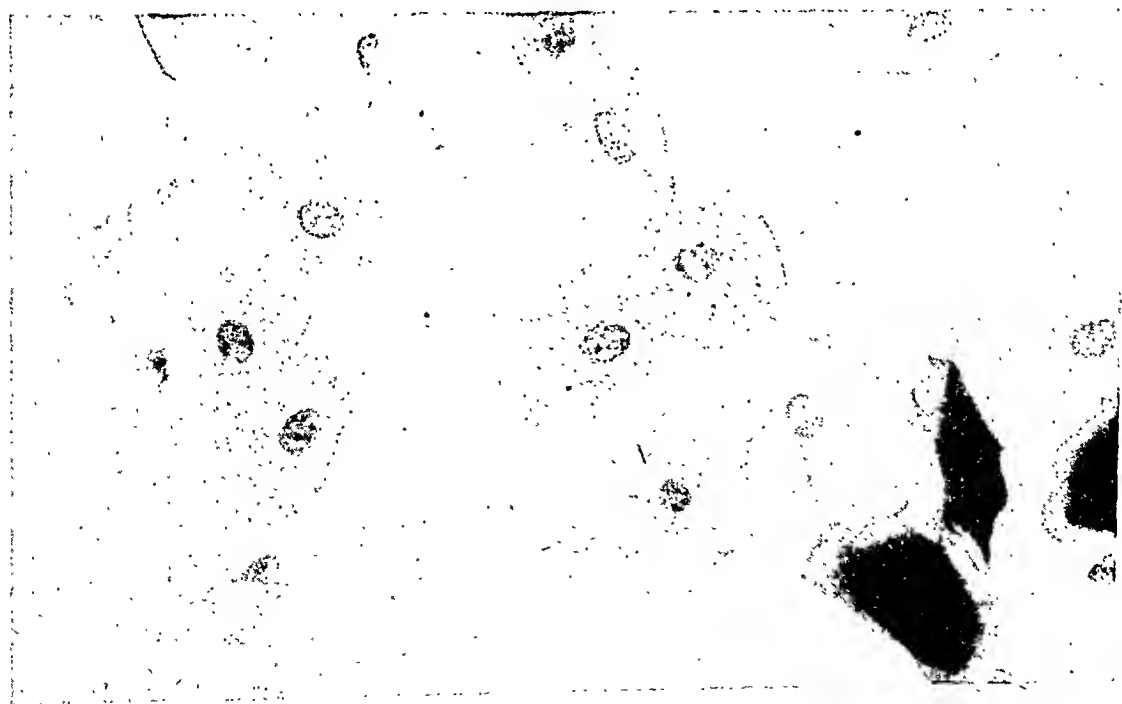


FIG. 4. Urethral smear of a hypogonadal male W.M., age 42; urinary 17-ketosteroid excretion, 2.1 mg./24 hrs.  $\times 600$ .

ject, semen analyses and measurements of urinary 17-ketosteroids were carried out. The amount of estrogen administered was found sufficient to produce an oligospermia, a marked change in the morphology of the sperms, and a marked reduction in the volume of the ejaculate; the 17-ketosteroids were lowered from 14.3 mg. to 8.1 mg./24 hrs. at the end of the fourth week of estrogen therapy.

### CONCLUSIONS

1. No significant differences are observed in the cytological picture of the urethral smear of normal and hypogonadal males.
2. Errors in interpretation of urethral smears may arise from drying of the smear prior to fixation. Procedures are described by which such drying can be prevented.

### ACKNOWLEDGMENT

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# EFFECTS OF STEROID HORMONES UPON THE DEVELOPMENTAL SEPARATION OF THE PREPUCE FROM THE GLANS PENIS<sup>a</sup>

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IT has been reported that by the tenth day after birth the normal separation of the prepuce from the glans penis has become complete enough "to allow mechanical retraction without danger of a tear."<sup>b</sup> However there are still those infants who are said to present clinical indications for circumcision. From experimental studies in animals it would seem that in these infants such therapeutical agents as androgen might be as effective as cutting.

In rodents, a developmental separation similar to the normal one of puberty may be induced experimentally by subjecting immature ground squirrels to subcutaneous injections of androgen (16). Similar observations in the rat and the mouse have been made (9, 14). It has also been found that in the rat the local application of androgen to the penis is followed by considerable growth of this organ (18).

The first objective of the present study was to determine whether androgen applied to the prepuce of rodents would cause a separation of the foreskin without causing an extensive growth of the penis. The second objective was to determine whether steroid hormones given in this manner to infants would accomplish the same purpose as circumcision without causing undesirable "side effects."

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<sup>a</sup> Aided by a grant from Ciba Pharmaceutical Products, Incorporated. The steroid hormones, Perandren and Di-ovocylin, were supplied by this firm through the courtesy of Ernst Oppenheimer, M.D.

<sup>b</sup> In the normal development and separation of the human prepuce, the embryonic primordium appears at about the 20-mm. stage (8). The primordium is a fold which consists of an outer sheet of ectoderm and an inner mass of mesoderm. As this fold enlarges it "flows" over the dorsum of the naked glans and eventually its right and left portions extend to the ventral aspect where they unite to produce the frenulum preputii. The epithelium of the prepuce fuses with that of the glans to form a solid layer, the so-called glandar lamella or balano-preputial lamella. Normal separation involves keratinization of this lamella and it begins about the sixth lunar month (2). Early manifestations are a desquamation near the tip of the glans and a formation of epithelial pearls at the site of the future collum glandis. Thereafter the keratinization proceeds from these points toward the center of the lamella. At birth the normal separation of the prepuce is still incomplete.

## ANIMALS

For the experiments in rodents the immature male ground squirrel (*Citellus tridecemlineatus*) was chosen. In this species the glans cannot be exposed manually until about the seventh month of postnatal life (December). The experiments were performed during October and early November.

A solution of testosterone propionate in propylene glycol was dropped on the prepuce by means of a blunted hypodermic needle (gauge 20). The concentration of the solution was such that each drop contained 0.2 mg. (20 mg. per cc.). Beginning on October 8, each of four animals was given one drop per day for a period of 27 days.

It was observed that on the fourth day of treatment there was considerable hypertrophy of the prepuce of each animal. On the seventh day the prepuce of one animal was large enough and free enough so that slight manual retraction exposed about half of the glans. Eventually the fore-skin of each animal could be easily and completely retracted, as in a normal adult (days 16, 19, 23, and 25).

TABLE 1. EFFECTS OF ANDROGEN UPON THE REPRODUCTIVE ORGANS OF GROUND SQUIRRELS\*

Animals	Body wt., Gm.	Weight of organs, mg.		
		Penis	Seminal vesicles	Prostate
Control	190	109.1	6.5	3.0
Experimental	220	187.6	22.7	9.2
Experimental	230	199.0	17.1	9.2

\* For additional explanation, see text.

On November 9, thirty-two days after the initial treatment and five days after the last, two of the experimental animals and one untreated control were autopsied. The data obtained at autopsy (Table 1) indicate that the androgen had caused growth of the penis, seminal vesicles and prostate. Yet all of these organs were much smaller than those of normal adult males. Stated differently, the weight of the organs of the experimental animals suggests that only a small amount of the androgen had entered the blood stream. As additional support for this view, data from an earlier paper may be cited: in 38 normal immature males the average weight of the seminal vesicles was 11.6 mg., while in 3 immature males receiving subcutaneous injections of testosterone propionate the average weight was 590.8 mg. (0.5 mg. of hormone per day for periods of 18 to 22 days) (17).

## INFANTS

During the several periods of study, all normal "mature" males born at a local hospital were used. These infants remained in the hospital throughout the period of treatment.

The procedure was as follows. On the day of birth the prepuce was carefully retracted manually so that not a single adhesion would be broken. The greatest transverse diameter of the glans thus exposed was measured by means of calipers which were graduated to tenths of a millimeter. Usually treatment was begun on the day of birth and continued daily for various periods of time. The nurses were instructed not to retract the prepuce of any baby in the hospital.

It did not seem worth while to obtain data on the length and diameter of the penis since at any time the dimensions would depend upon the amount of blood in this erectile organ. Also, since spontaneous erections were observed in several of the controls at the time when the diaper was being changed, it was decided not to keep a record of the number of erections per baby.

The usual method of treatment was for one of the authors (L.J.W.) 1) to wrap the prepuce loosely around the mouth of a hypodermic syringe (0.25 cc.), 2) to press the plunger until 0.05 cc. of the solution of hormone had entered the space between prepuce and glans (frequently it also entered the urethra), 3) to remove the syringe and then use two fingers and thumb for spreading the solution over the entire prepuce and 4) to allow about a minute for partial evaporation of the solvent before permitting the prepuce to touch the diaper. When the treatments were made twice daily, they were spaced about 12 hours apart.

A second method was used only in the case of nine infants (Group D, Table 3). Nurses were instructed to use a calibrated medicine dropper which delivered 44 drops per cc. They were also told to let one drop fall upon the prepuce at the time of each feeding. In order to minimize the chances of error, they were asked to treat all male infants in the hospital until further notice.

Usually on the day after the last application, the prepuce of each infant was retracted by one of the authors (C.L.) and the glans penis was measured by the other (L.J.W.). With respect to procedure, the prepuce was retracted manually as far as possible without tearing adhesions (if present) and the greatest transverse diameter of the portion of glans in view was measured and recorded. Then the tip of a closed hemostat was placed in the space between prepuce and glans and the foreskin was gently stretched by opening the jaws of the instrument. If, because of adhesions, it was still impossible to complete the retraction manually without causing trauma, such adhesions were broken by means of a probe. Finally, as soon as the

retraction had been completed, the glans was returned to its sheath.

Regarding the controls, it may be noted that in all except four of them (71%) a complete retraction was accomplished without cutting (Table 2). That this was possible in certain controls is evidence against the notion that a firm union of glans and prepuce at birth is a reliable indication for circumcision (Infants 2, 3, 7, 10, 11 and 12). At the time of retraction by means of instruments, exactly half of the controls showed adhesions that were quite firm. When bleeding occurred after adhesions had been broken, there

TABLE 2. OBSERVATIONS IN INFANTS (CONTROL SERIES)

Infants	Blank applications (days) <sup>a</sup>	Age at retraction (days)	Body weight (gm.)		Glans free of adhesions (diameter, mm.)		Retraction by means of hemostat and probe	Adhesions <sup>b</sup>	Bleeding after breaking adhesions
			At birth	At retraction	At birth	At retraction			
1	20 P	32	2837	3574	3.5	5.0	Complete	++	0
2	0	28	3603	4057	0.0	3.8	Complete	++	0
3	0	24	2894	2997	0.0	4.0	Complete	+	0
4	0	18	3348	3433	5.7	7.5	Complete	+++	+
5	0	18	3660	3774	0.0	0.0	Partial	+++	+
6	0	17	3221	3206	2.9	4.1	Complete	++	+
7	0	17	3632	3916	0.0	1.8	Complete	++	+
8	14 P	15	3376	3376	0.0	0.0	Contraindicated	+++	+
9	0	15	2497	2951	1.5	3.6	Contraindicated	+++	+
10	13 P	14	3859	3859	0.0	1.3	Complete	+++	+
11	12 S	13	3462	3462	0.0	4.0	Complete	+++	+
12	0	12	3860	3774	0.0	3.6	Complete	++	0
13	0	12	3539	3617	3.0	3.4	Complete	++	0
14	0	8	2922	2951	0.0	0.0	Contraindicated	+++	+

<sup>a</sup> Propylene glycol (P) or sesame oil (S).

<sup>b</sup> + + +, quite firm; ++, moderately firm; +, slightly firm.

was generally a single drop of blood which came from the region of the frenulum. In Infant 5, the bleeding began as soon as the jaws of the hemostat had been spread to 3.7 mm. and the retraction was not completed because the prepuce was so thin and unyielding. In Infants 8 and 14, a spread of 3.5 mm. caused bleeding, while in Infant 9, a spread of 6.0 mm. caused it.

In the experimental series (Table 3), complete retraction was possible in 22 of the 27 cases (81%). Five of the infants showed no adhesions. Of those having adhesions, Infant 36 was the only one in whom they were quite firm. It would seem, then, that one effect of the hormones was a softening of the adhesions. In most cases as soon as the hemostat had been spread and the prepuce had been pulled back as far as possible without tearing, a ring of smegma was observed at the junction of prepuce and glans. Doubt-

TABLE 3. OBSERVATIONS IN INFANTS (EXPERIMENTAL SERIES)

Groups	In- fants	Appli- cation of hor- mones (days)	Age at retrac- tion (days)	Body weight (gm.)		Glans free of adhesions (diameter, mm.)		Retraction by means of hemostat and probe	Adhe- sions <sup>a</sup>	Bleeding after breaking adhe- sions
				At birth	At retrac- tion	At birth	At retrac- tion			
<i>Androgen in propylene glycol, one application per day (1.0 mg. daily)</i>										
A	15	25	26	3036	3291	0.0	7.3	Complete	0	0
	16	23	27	3447	4001	0.0	2.5	Complete	+	+
	17	20	21	3774	3887	2.4	12.6	Complete	0	0
	18	20	25	2781	3036	4.1	7.2	Complete	+	0
<i>Androgen in propylene glycol, two applications per day (2.0 mg. daily)</i>										
B	19	17	23	3348	3603	0.0	3.4	Almost complete	0	0
	20	17	22	3802	4129	0.0	12.6	Complete	+	0
	21	16	17	3830	3774	0.0	5.6	Complete	+	+
	22	15	16	3334	3603	0.0	4.7	Complete	++	+
	23	14	15	3518	3660	2.0	6.5	Almost complete	+	0
	24	13	14	4731	4540	4.0	7.0	Complete	+	0
<i>Androgen in sesame oil, two applications per day (2.5 mg. daily)</i>										
C	25	14	15	2873	3235	5.7	9.0	Complete	+	0
	26	13	14	3646	3745	2.7	13.5	Complete	0	0
	27	13	14	3873	4001	0.0	9.2	Complete	+	0
	28	12	13	3901	3916	2.7	5.7	Complete	+	+
	29	12	13	3462	3405	0.0	9.0	Complete	0	0
	30	10	11	3008	3036	0.0	3.0	Complete	+	+
<i>Androgen in propylene glycol, six applications (drops) per day (3.0 mg. daily)</i>										
D	31	13	24	3547	3916	0.0	5.6	Partial	++	+
	32	13	21	3121	3178	0.0	7.1	Complete	+	0
	33	12	28	3235	3405	0.0	6.0	Complete	++	+
	34	12	23	3689	3887	0.0	2.3	Partial	++	+
	35	12	22	2922	3234	0.0	4.8	Complete	+	0
	36	12	22	3405	3376	0.0	3.5	Contraindicated	+++	
	37	12	21	3192	3674	0.0	5.9	Complete	+	+
	38	12	17	3121	3163	0.0	4.0	Complete	+	0
	39	8	30	3348	3745	2.3	6.2	Complete	+	+
<i>Estrogen in sesame oil, two applications per day (0.5 mg. daily)</i>										
E	40	8	9	3972	3603	2.3	5.8	Complete	+	+
	41	6	7	3405	3178	2.3	2.5	Complete	+	0

a. +++, quite firm; ++, moderately firm; +, slightly firm.

less this material must have originated from the breakdown (cornification) of the distal portion of the glandular lamella.<sup>b</sup>

Retraction was easiest in the infants of Group A. While it seemed that

the glans was somewhat larger than that of controls, the prepuce was so large and pliable that there was little difficulty in completing the retraction. In Infants 15 and 17, the retraction was readily completed without instruments. These observations suggest that the application of 1.0 mg. once daily for three weeks is more effective than the application of 1.25 mg. twice daily for two weeks (Groups A and C).

In Groups B and C, it seemed that the glans had grown as rapidly as the prepuce. From the standpoint of retraction, this growth of the glans was undesirable. In fact, in Infants 19 and 23, it was decided not to try to complete the retraction because the glans was disproportionately large in comparison with the prepuce.

Yet the growth of the penis had not been undesirably rapid. Infants 18 and 20 were observed again on the 36th and 39th day after birth, respectively, and it was noted that the penis was not undesirably large.

From memory and general impression, the frequency of erections in the experimental series was about the same as that in the control series. Infant 22, however, was exceptional in that his penis became erected nearly every time an application was made.

In Group D the treatments were begun at birth but were discontinued several days before the retraction. The data would seem to indicate that applying 3.0 mg. per day for two weeks was less effective than applying 1.0 mg. per day for three weeks (Groups D and A). However in Group D it is possible that the effects of treatment had disappeared in part during the interval of non-treatment. Certainly at the time of retraction the glans was not disproportionately large.

Estrogen also caused a loosening of adhesions but failed to cause any detectable growth of the penis (Group E). Only one control showed adhesions that were slightly firm and this control was 26 days of age (Infant 3). Presumably the estrogen acted by causing growth and cornification of the balano-preputial epithelium. In this connection it may be mentioned that when estrogen acts in this manner upon the vaginal epithelium of immature rats it causes the developmental opening of the vagina (1).

## DISCUSSION

The question arises as to why the androgen induced a complete separation of the prepuce in only twenty per cent of the infants of Groups A to D (Infants 15, 17, 19, 26 and 29). While the data are not adequate to permit a definite answer, they suggest that the main factor was the relatively short periods of treatment. Certainly in the seven infants who received treatment for periods of 16 to 25 days there was an absence of adhesions in a much higher percentage of cases (43%). It may be recalled that in the individual



experimental animals the disappearance of adhesions occurred on days 16, 19, 23 and 25.

The data also suggest that applying androgen in sesame oil was equally as effective as applying it in propylene glycol. In appraising the relative effectiveness of these methods, two important variables must be kept in mind, namely, the number of applications and the quantity of hormone.

Absence of marked growth of the penis suggests that a relatively small quantity of the androgen had entered the circulation. Doubtless only a small quantity of the estrogen had entered it. Consequently it is reasonable to suppose that these steroid hormones had not caused any undesirable effects upon skeleton and internal reproductive organs.\*

It is not the purpose of this paper to discuss the controversial problem of circumcision. The physician who more or less routinely practices this procedure would not find the use of androgen especially helpful. On the other hand we do not subscribe to such routine and our opinion is shared by many clinicians. There are two distinct purposes of circumcision. One is to permit complete retraction of the prepuce and the other is to remove part of an excessively long prepuce. We have no evidence that androgen affects the length of the prepuce but it does reduce the adhesions which commonly hinder normal retraction. It was possible to retract the foreskins of all treated babies in groups A, B and C satisfactorily, yet it was not possible in 29 per cent of a control group of comparable size.

Two difficulties arise when this method is applied clinically. The first of these is time. The data suggest that treatment for 20 to 25 days produced better results than did shorter periods of treatment. Rarely do newborn infants remain in the hospital for more than 10-15 days at present. While it might be possible to continue such treatments in the home, our data suggest that skill and care in the topical application are of considerable importance.<sup>d</sup> The second difficulty, of minor importance, was the tendency of

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\* Any notion that our treatment of these infants might have led to premature development of the skeleton deserves little more than passing attention. The fetus is normally exposed to those estrogenic and androgenic substances which are in the fluids of pregnancy (4, 6). Also the quantity of hormone and the duration of treatment were negligible in comparison with those which have been reported to accelerate the development of the skeleton (10) (cf. effects of gonadotropin) (3). Nor is there reason to suspect that our treatments caused permanent effects upon spermatogenesis. While steroid hormones may inhibit spermatogenesis by acting upon the anterior hypophysis (12), the effects are reported to be more or less temporary (7, 11, 19). In 1942 Moore and Morgan concluded that "The effects of androgens upon the testis remain an open question" (13). Subsequently it has been reported that in hypophysectomized animals the injection of androgen will maintain spermatogenesis (5), will restore spermatogenesis (15) and will even induce the precocious formation of spermatozoa (17).

<sup>d</sup> It is likely that androgen in the form of an ointment could be satisfactorily applied

the glans to enlarge. While this was of no clinical significance it did tend to offset some of the advantages obtained by the reduction of adhesions.

Finally, the direct application of androgen is apparently of limited value in those very infants who present clinical indications for circumcision.

### SUMMARY

The topical application of testosterone propionate to the prepuce of immature ground squirrels induced a developmental separation of the prepuce from the glans penis. This treatment caused only slight growth of such accessory reproductive organs as the penis, the seminal vesicles and the prostate.

Similar treatment of infants caused a complete separation of the prepuce in 5 of the 25 cases (20%). In most of the other cases this treatment led to a softening of those adhesions which commonly hinder the normal retraction of the foreskin. Likewise the topical application of estradiol dipropionate was followed by a loosening of such adhesions. These steroid hormones did not cause any undesirable "side effects."

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to the prepuce of an infant by the parents. We did not use this form of treatment because testosterone propionate is much more soluble in oil than in an ointment base. Incidentally, applying androgen in propylene glycol is less satisfactory than applying it in oil since micturition would remove the former solvent more readily than the latter.

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# EXCRETION OF 17-KETOSTEROIDS IN ANKYLOSING SPONDYLARTHROSIS AND IN RHEUMATOID ARTHRITIS: A PRELIMINARY REPORT

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**B**ECAUSE of the peculiar sex distribution of various types of arthritis a study of the urinary 17-ketosteroid excretion was made. No study of this type has previously been reported in arthritic patients. It has long been observed clinically that in ankylosing spondylarthrosis (Marie-Strümpell type) there is a great preponderance of males over females. "The highest incidence of females recorded has been 10 per cent" (1). In rheumatoid arthritis, there is a less marked but important preponderance in incidence of the disease in females over males: "Females are much more frequently affected (3:1) than males" (3). In studies of follicle-stimulating-hormone excretion in women under 40 years of age with rheumatoid arthritis Sjövall recently concluded that 21 per cent showed ovarian insufficiency (6).

The 17-ketosteroids are a group of compounds which form the metabolic end-products of steroids originating in the adrenal cortex of the female and in both the adrenal cortex and the gonads of the male. Normally, approximately 90 per cent consists of androsterone and its inactive isomer, 3-hydroxy-etiocholanone, the remainder being mostly dehydro-iso-androsterone which is weakly effective as a male sex hormone. The excretion in normal males varies between 8 and 28 milligrams in 24 hours, average, 14 milligrams; in normal females the limits are 5 to 18 milligrams in 24 hours, average, 9 to 10 milligrams (4). The excretion of 17-ketosteroids is lowered in Addison's disease and in hypothyroidism and moderately increased in hirsutism of females, in masculinizing ovarian tumors, and in Cushing's syndrome. It is, in general, greatly increased in hyperplasia and in carcinoma of the adrenal cortex where excretion values can rise to more than 300 milligrams in 24 hours, although exceptions to this have been noted (2).

In our series of 13 cases of ankylosing spondylarthrosis all patients were males, and all showed characteristic x-ray changes in the spine; several also showed some changes in the hips. The small joints of the extremities

were not affected in these patients. In this group 10 cases were active, 1 questionably active, and 2 inactive as judged by sedimentation rate and physical findings. The average age for the group was 39.4 years, range, 25 to 43 years. The average duration of symptoms was 10.6 years, range, 3 to 26 years. Two patients had received previous x-ray therapy.

In our series of 11 cases of rheumatoid arthritis all patients were females, and the disease was limited principally to the joints of the extremities. In this group the average age was 40.4 years, range, 25 to 52 years. Eight of

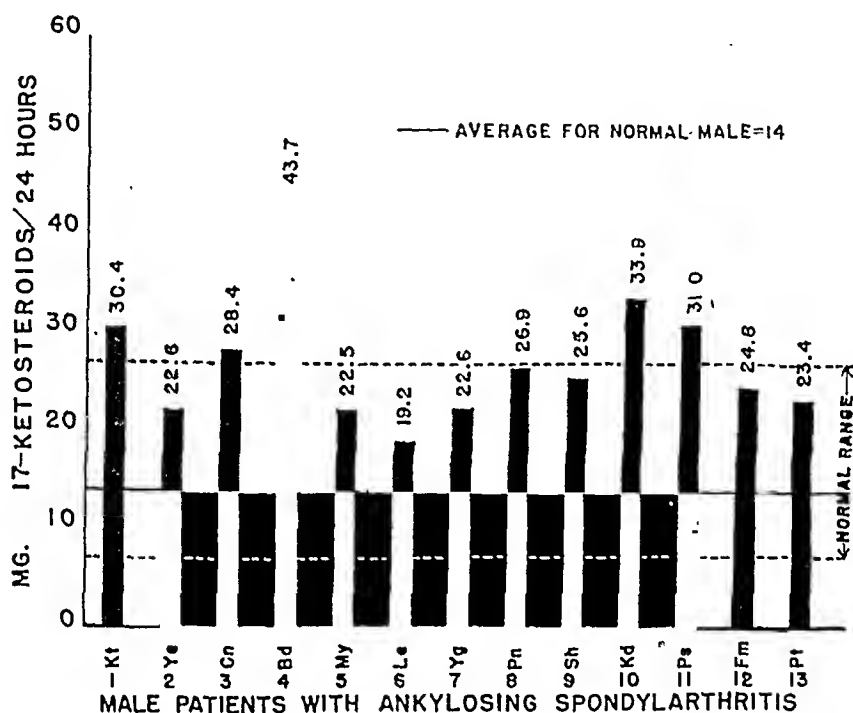


FIG. 1

the 11 patients were receiving chrysotherapy at the time the determinations were made. Ten of the 11 cases were active as judged by sedimentation rate and physical findings, and the average duration of symptoms was 5 years.

#### METHOD

The method used by us was an adaptation of that of Robbie and Gibson (5). Twenty-four-hour specimens of urine were collected and the volume measured. To 250 cc. of urine were added 25 cc. of concentrated hydrochloric acid and the mixture boiled at a reflux for 7 minutes. After cooling, 60 cc. of carbon tetrachloride were added and the boiling continued on a water bath for 10 minutes. The carbon tetrachloride layer was washed twice with water, twice with 10 per cent sodium hydroxide, and again with water until neutral. After evaporation *in vacuo* the residue was taken up in

10 cc. of absolute alcohol. For the colorimetric measurement, 1 cc. of the extract solution was added to 4 cc. of 95 per cent alcohol, 1 cc. of 2 per cent m-dinitrobenzene in absolute alcohol, and 1 cc. of 15 per cent aqueous potassium hydroxide. The color was read after 90 minutes in a Klett-Summerson colorimeter and compared with a standard prepared from crystalline androsterone. Blanks were run to correct for urinary pigments and for the m-dinitrobenzene color. The 17-ketosteroid excretion was then computed for the total 24-hour volume. Values obtained by this method

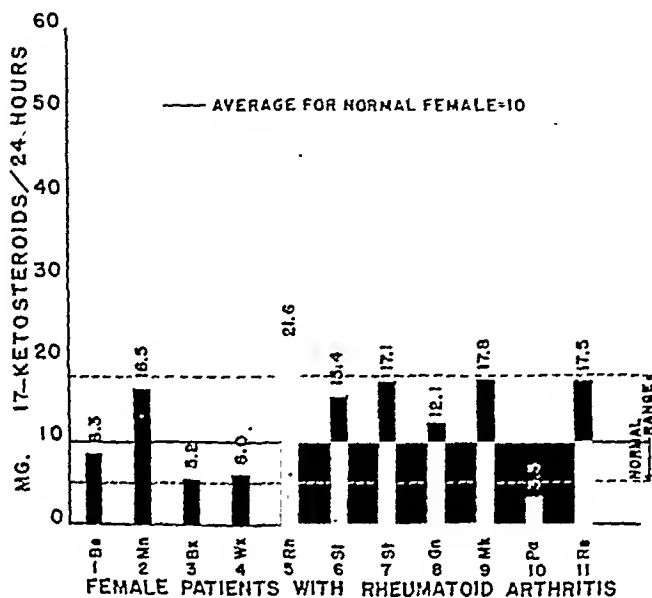


FIG. 2

show excretion of normal adult males varies between 8 and 28 milligrams in 24 hours, average, 14 milligrams; for normal females the limits are 5 to 8 milligrams in 24 hours, average 9 to 10 milligrams.

### RESULTS

Figure 1 shows the urinary excretion of 17-ketosteroids in 13 patients with ankylosing spondylarthritis. It is seen that the range is from 19.2 milligrams to 43.7 milligrams in 24 hours, the average for the group, 27.3 mg. The 17-ketosteroid levels in this series group themselves around the upper limit of normal for males.

Figure 2 shows the urinary excretion of 17-ketosteroids in 11 female patients with rheumatoid arthritis. The range in this group is from 3.5 to 21.6 milligrams in 24 hours, average, 12.8 milligrams. The 17-ketosteroid

levels for these patients are scattered through the normal range for females.

A subsequent report will present results of 17-ketosteroid determinations in patients with spondylarthritis during and after treatment with x-ray and in patients with rheumatoid arthritis after chrysotherapy. Similar determinations are being made on patients with gout and on males with rheumatoid arthritis, and attempts will be made to fractionate the ketosteroids when their levels are elevated.

### CONCLUSIONS

The urinary excretion of 17-ketosteroids in 13 male patients with ankylosing spondylarthritis (Marie-Strümpell type) averaged 27.3 milligrams in 24 hours as compared with an average of 14 milligrams for normal males. In 11 females with rheumatoid arthritis the average urinary excretion of 17-ketosteroids was 12.8 mg. as compared with an average value of 10 mg. for normal females. Thus there seems to be a trend to greater excretion of 17-ketosteroids in ankylosing spondylarthritis, a disease largely confined to males; whereas, in typical polyarticular rheumatoid arthritis in females, this tendency is not demonstrated.

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# INSULIN REGULATION IN ONE HUNDRED AND TWENTY-SIX DIABETIC CHILDREN\*

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**T**HIS report concerns 126 campers during July and August, 1946, at Camp Nyda, the summer camp for diabetic children conducted by the New York Diabetes Association. Each child remained four weeks. The insulin dosage of the precamp treatment was maintained unless the precamp insulin did not control the diabetes or more than morning and evening injections of insulin were scheduled. When the type of insulin was changed, the first shift was made to the 1 part P and 2 parts R—advocated by

TABLE I. TYPES OF INSULIN ADVISED BY CLINICS AND PRIVATE PHYSICIANS FOR THE 126 CHILDREN BEFORE THEY WERE ADMITTED TO THE CAMP AND MODIFICATIONS FOUND ADVANTAGEOUS WHILE AT CAMP (WHEN CHANGES WERE MADE PREFERENCE WAS GIVEN TO THE 1 PART P TO 2 PARTS R MIXTURE)

Insulin	No. cases precamp	No. cases on discharge	No. cases changed after 3 to 4 weeks at camp
P	26	27	1 more
R	14	0	14 less
G	10	10	no change
separate injections: P & R	44	28	16 less
mixtures: P & R	25	54	29 more
separate injections: P & G	4	4	no change
separate injections: R & G	2	2	no change
none	1	1	no change

Colwell (1) as the most nearly universally applicable insulin. Consequently, our discharge types of insulin are weighted in favor of the 1-to-2 mixture.

The standards of diabetes control at Camp Nyda were a sugar free urine and the avoidance of hypoglycemic reactions. These objectives were achieved in nearly all children for most of their stay. The insulin administered at the end of three or four weeks represents the insulin that was finally considered successful in controlling the diabetes under camp conditions of diet and exercise.

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\* In this presentation, protamine zinc insulin is designated by the letter "P," unmodified (regular) and crystalline insulin by "R," globin insulin with zinc by "G." From Camp NYDA conducted by the New York Diabetes Association, Inc.



A summary of the admission and the discharge insulins is given in Table I. Several conclusions from this table are obvious. P or G as the only insulin used was satisfactory in many cases. R alone was a failure in every child receiving this insulin. Separate injections of P and R were not as successful as the mixtures of P and R. Mixtures of P and R proved acceptable in nearly all instances when shifts in the type of insulin were deemed necessary, though in two cases a change to P alone from the P and R mixtures proved to be advantageous. As mentioned above, the mixtures of P and R were immediately resorted to for modification of the kind of insulin, which accounts for the large increase in this type of insulin on discharge. The administration of G and R, and of G and P, gave good results in a few instances. However, the disadvantage of two injections instead of one has to be taken into consideration.

### Protamine Zinz Insulin as the Sole Insulin

On admission, 26 children received P, and no other insulin (Table II). In 14, the dose was above 30 units and in 6, above 40. It was considered expedient to shift from P to the 1-to-2 mixture in only 4 instances. This is remarkable because it has been stated over and over again that comparatively few diabetics do well on P alone, and, as stated recently by Palmer, (2) scarcely ever when the dose exceeds 30 units.

An accentuation of the immediately preceding statements is found in Table III which shows that several cases gave evidence of better control of their diabetes with P alone than with combinations of P and R. After several weeks' observation a combination of Tables II and III showed that the level of the P dose had not changed a great deal since 14 children were receiving more than 30 units and 6, more than 40, the identical numbers for the higher doses of P as on admission.

Probably there could have been many more patients successfully managed with P alone, but as stated previously, in making shifts of the type of insulin, the 1-part P to 2-parts R insulin was resorted to in routine fashion.

### Unmodified (or Crystalline) Insulin as the Sole Insulin

There were 14 children receiving R as the only insulin according to their precamp record (Table IV). The doses varied from 5 to 70 units by single or multiple injections. When injections were given more often than twice a day (before breakfast and supper) then other types of insulin calling for the administration of one daily dose were substituted. Also, when R alone proved to be unsatisfactory for the control of the diabetes, changes were made. The final result was, as may be noted in Table IV, that R as the sole insulin was rejected in every instance, and other forms of insulin proved to be more suitable for the campers.



TABLE IV. CASES RECEIVING R ONLY ON ADMISSION. IT WAS FOUND ADVISABLE TO CHANGE THE TYPE OF INSULIN IN EACH OF THESE 14 CHILDREN

Total R units precamp	Replaced at camp by	
R 5 units	P 10 units	
R 15	1 to 2 mixture*	18 units
R 15	1 to 2 mixture	15 units
R 20	1 to 2 mixture	24 units
R 26	1 to 2 mixture	24 units
R 27	1 to 2 mixture	18 units
R 35	R 15 P 10 mixture	25 units
R 42	1 to 2 mixture	30 units
R 42	1 to 2 mixture	30 units
R 55	1 to 2 mixture	42 units
R 60	1 to 2 mixture	42 units
R 60	1 to 2 mixture	52 units
R 65	1 to 2 mixture	42 units
R 70	1 to 2 mixture	48 units

\* 1 to 2 mixture is a mixture containing 1 part P to 2 parts R.

TABLE V. 16 CASES RECEIVING G ON ADMISSION TO THE CAMP. IN NOT A SINGLE INSTANCE WAS IT FOUND NECESSARY TO CHANGE THE TYPE OF INSULIN

Total G units precamp			Modification in 3 to 4 weeks		
G 12 units			G 12 units		
G 13			G 32		
G 20			G 5		
G 30			G 26		
G 40			G 36		
G 40			G 37		
G 50			G 40		
G 52			G 46		
G 60			G 45		
G 60			G 56		
-----					
G 10	P 40	separate injections	G 12	P 30	separate injections
G 28	P 16	separate injections	G 26	P 18	separate injections
G 36	P 20	separate injections	G 14	P 36	separate injections
G 36	P 54	separate injections	G 20	P 30	separate injections
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G 20	R 10	separate injections	G 30	R 9	separate injections
G 34	R 14	separate injections	G 35	R 16	separate injections

### Globin Insulin with Zinc

16 cases receiving G on admission (Table V) were continued on the same insulin throughout their stay at camp. The control of the diabetes with G was so satisfactory that we felt it would be worth a more extended trial when other insulins proved inadequate. However, we had committed ourselves to give 1 part P to 2 parts R mixture, and did not deem it wise to switch policies during the two-month period. The more extensive use of G may be taken up in a subsequent session of the Camp. The diet regime with snacks between breakfast and lunch, between lunch and dinner, and at bedtime, besides the three regular meals, is probably a system of feeding particularly adapted to the use of G, and for that matter to the requirements of the 1 part P, 2 parts R mixture, which has an effect a good deal like G.

### Combinations of P and R

P and R in combination are administered by three methods: by separate injections, by set mixtures in which the P and R are mixed in a stock vial, and by mixing the P and R in the syringe just before injection which may be given the name "adjustable mixture."

### R and P by Separate Injections

There were 44 children, more than a third, on such a schedule, on admission to the camp. This indicates the popularity of using insulin in this way. The amount of insulin given varied enormously. The smallest dose was R 7-P 6, the largest, R 46-P 70. Under camp conditions, this mode of administering insulin was not very successful, as can be gathered from the fact that out of 44 diabetics, this type of insulin therapy was discontinued in 16 (Table I).

### Set Insulin Mixtures

Precamp procedures included only 4 of these, two on a mixture of 1 part P to 2 parts R, and two on 7 parts P to 10 parts R. These four cases continued on the same mixture throughout their stay at camp. When other forms of insulin proved unsatisfactory for any reason, transfer was made to a mixture of 1 part P to 2 parts R. This is the proportion advocated by Colwell (1) as the most universally applicable combination of P and R, so that there were 29 children receiving this type of insulin after 3 to 4 weeks' stay at camp (Table VI).

### Adjustable Mixtures of P and R

These mixtures, made in one syringe, were used by 21 children on admission to the camp. It may be the mechanical difficulty of measuring two

insulins in one syringe that accounts for this comparatively small number. In our experience, the combination of the two insulins in one syringe was a much more satisfactory method of dispensing insulin than when P and R were given by separate injections. A total of 11 cases receiving separate injections on admission to the camp were benefited by changing over to adjustable mixtures. In all, there were 25 cases receiving adjustable mixtures after 3 to 4 weeks' sojourn at Camp Nyda. Of these, the highest total units was 74, the lowest 18.

TABLE VI. CASES ON SET INSULIN MIXTURES AFTER BEING AT CAMP AND THE TYPE OF INSULIN REPLACED

No. of cases	
4	set insulin mixtures on admission
12	replacing R alone
4	replacing P alone
5	replacing adjustable mixtures
4	replacing separate injections of P and R
<hr/>	
29	Total on set mixtures after 3 to 4 weeks at camp

The proportion of P to R in these adjustable mixtures is of interest, since it has often been stated that the amount of P cannot exceed that of R, and prove to be of value. However, in many instances, an excess of P over R proved valuable both under home and camp conditions. (See Table VII.)

TABLE VII. CASES RECEIVING ADJUSTABLE MIXTURES IN WHICH P EXCEEDED R EITHER ON ADMISSION OR AFTER STAY AT CAMP. THIS BY SOME HAS BEEN CONSIDERED A USELESS PROCEDURE, BUT IT APPARENTLY HAS A WIDE APPLICATION. IN NONE OF THE INSTANCES WAS THE DOSE OF INSULIN SO SMALL THAT THE PROPORTION OF P TO R WAS A MATTER OF INDIFFERENCE

Units of P and R mixed in syringe		Total units	Units excess P over R
P 24	R 14	38 units	10 units
P 25	R 10	35	15
P 28	R 18	46	10
P 30	R 10	40	20
P 31	R 10	41	21
P 32	R 29	61	3
P 32	R 20	52	12
P 42	R 15	57	27
P 45	R 15	60	30
P 46	R 20	66	26
P 80	R 24	104	56

## SUMMARY AND CONCLUSIONS

The insulin regulation of 126 diabetic children was studied at Camp Nyda during July and August, 1946.

Of the three types of insulin, R, P, and G, when given as the sole insulin, only P and G were satisfactory; in many instances R was a distinct failure. The large doses of P alone, applicable in some instances, are noteworthy; on discharge from the camp 14 children were receiving more than 30 units of P only, and 6 were receiving more than 40 units.

P and R by separate injections had to be replaced by other forms of insulin in many cases. Besides the disadvantage of a double injection, this form of insulin therapy proved less satisfactory than the insulin mixtures made in one syringe.

Insulin mixtures of P and R prepared in one syringe proved effective. Where insulin changes were believed advisable, the routine switch was made to a mixture of 1 part P to 2 parts R advocated by Colwell as the most generally applicable insulin. However, we believe that G would have yielded equally good results. Although the idea that P should not exceed the amount of R in one-syringe mixtures is prevalent, it was shown that on an empirical basis, P was often greater in amount than R for optimal results.

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# THYROTOXICOSIS COMPLICATED BY SEVERE IODISM: PREPARATION FOR SURGERY WITH PROPYL-THIOURACIL

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AS commonly used in the preoperative management of thyrotoxicosis, Lugol's solution is generally considered to have no harmful potentialities. Few instances of severe toxic reactions to this drug are reported in the literature. With the exception of two cases in a series reported by Barker and Wood (3) in 1940, all the reported episodes were mild to moderate in degree. As further evidence that Lugol's solution even in small doses may, on occasion, be a dangerously toxic drug, a case of almost fatal poisoning is here presented.

Case Report: H.B., a negress, age 22, was admitted to the medical service on May 4, 1946, with the classical complaints of hyperthyroidism. Her symptoms were nervousness, insomnia, marked fatigability and irritability, loss of weight despite an excellent appetite, intermittent diarrhea, and intolerance to heat with excessive perspiration. She had noted a mass in the neck, gradually increasing in size, associated with some difficulty in swallowing, and shortness of breath after mild exercise. In addition, her menses, which had been regular since their onset at the age of thirteen, had become markedly irregular in the past two years.

Most symptoms were of one year's duration and progressing in severity, but the loss of weight was most marked in the preceding three months, during which time the patient estimated that she lost 60 lbs. She had been quite obese, weighing 235 lbs. at one time. On admission her weight was 165 lbs. She had received no previous medical care for this condition.

Physical examination revealed a well-developed and well-nourished patient, somewhat nervous, but not acutely ill. Bilateral exophthalmos was present, with definite lid lag and some difficulty of convergence of the eyes. The thyroid gland was enlarged bilaterally, soft and not nodular. No bruits were heard over the gland.

Examination of the heart revealed a thrusting apex beat, but no thrills were palpated. A grade 2 systolic murmur was heard best at the apex, and was transmitted to the left axilla. Fluoroscopy gave evidence of hypertrophy of the left ventricle.

The lungs were normal to percussion and auscultation. The abdomen was entirely normal; liver and spleen were not palpable. The extremities showed only a fine tremor of the hands, which were moist and warm. In view of the history and findings, a diagnosis of hyperthyroidism was made.

The results of laboratory procedures, done on admission, were as follows: RBC—3.82 million, Hb—57%, WBC—4,700 with 52% polys, 37% lymphocytes, 7% monocytes, and 3% eosinophiles. The urine was entirely normal. Blood chemistry showed an NPN

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of 42, fasting glucose of 80. Blood serology was negative. The BMR was +62 per cent.

The patient was placed on a high caloric diet with vitamin supplements. Barbiturates were administered for sedation, and on May 6 the patient was started on Lugol's solution, Minims X, t.i.d. The advisability of using thiouracil in this case was fully considered, but it was concluded that the patient could be more quickly prepared for surgery, with less likelihood of a toxic reaction, by means of the Lugol's solution.

On this date, May 6, an EKG showed a sinus tachycardia and nonspecific ST-T changes, interpreted as being compatible with hyperthyroidism.

The patient responded well to this therapeutic regimen. She became much less nervous and irritable, and there was some slowing of the heart rate. The basal metabolism on May 11, five days after starting iodine, was plus 46 per cent, and by May 18 it had dropped to plus 26 per cent. However, she continued to lose weight during this period, and went from 165 lbs. on admission, to 152 lbs. on May 18.

On May 14, the patient complained of mild generalized pruritis, most marked on the neck. A mild acne of the face, back and chest had been noted on admission. Superimposed on this was a fine macular rash over the same areas, first noted on May 15. It faded within 24 hours, although mild pruritis persisted.

On May 18, the patient complained of a burning sensation and tearing of both eyes. During the evening she developed symptoms of coryza and a sore throat. Examination on the following morning, May 19, revealed a severe bilateral conjunctivitis, with marked dacryorrhea; there were also a profuse sialorrhea, and marked congestion of the nasal and pharyngeal mucosa. The tonsillar areas were covered with a thin white exudate. At this time the administration of the Lugol's solution was stopped because iodism was suspected.

On May 19, the patient's temperature ranged from 101-103.4° F. She continued to run an irregular fever, with a maximum of 104° F. until June 1.

Coincident with the rise in temperature and findings as above, the patient was started on penicillin, 20,000 U. every 3 hours. She was also given intravenous infusions of glucose in saline, and symptomatic therapy. Despite this, the condition became worse. Her conjunctivitis became purulent in type, and there were desquamating and bleeding lesions of the lips, buccal mucosa and tonsillar area, with marked cervical node enlargement. She further developed a discrete, reddish, maculopapular rash, generalized over the body and prominent on the hands and feet, with moderate pruritis. The diagnosis of iodine toxicity could now be established. However, the use of the barbiturates was also stopped.

By the evening of May 20, this sudden and fulminating toxic reaction had extended to the tracheobronchial tree. There were now signs of dyspnea, a dry cough, and diffuse wheezy and crackling rales in both lungs. The patient was placed in an oxygen tent. Her WBC at this time was 11,000.

The following day the WBC was 9,650 with 77% polys, 15% lymphs, 8% monocytes, and no eosinophiles. The RBC was 4.52 million with 84% Hb. Blood culture was negative. Throat cultures showed a variety of organisms, but were negative for diphtheria bacilli.

On May 22 the patient was toxic and exhausted, and markedly dyspneic. She was now coughing up huge amounts of a pale pink, watery sputum. Loud moist rales were heard in both lungs. In addition the patient now had frankly bloody urine, and the vaginal mucosa showed the same type of desquamating lesions as were apparent in the mouth.

A dermatological consultant saw the patient, and stated that the skin rash was of an erythema multiforme type, presumably on a toxic basis. Penicillin dosage was now stepped up to 40,000 U. every 3 hrs. The following day her general condition was es-



essentially unchanged. The skin lesions were larger, and covered her entire body, and were associated with marked tenderness of the palms of the hands and soles of the feet. Repeat blood serology was again negative.

Late that night, the patient had an episode of severe dyspnea, with inspiratory stridor and expectoration of profuse and tenaceous pink sputum. The trachea was repeatedly aspirated, and much thick mucous material was sucked up, with improvement in breathing.

The following day she was better, with less difficulty in breathing, although the profusely productive cough continued. An x-ray examination of the chest was negative. WBC was 7,400. However her RBC had fallen to 3.46 million, and Hb was 51%. Urinalysis showed a thickish, dark brown urine, with 4 plus albumen, many RBC and WBC, and many bacteria. There was no hemoglobinuria.

She continued to improve, and on May 26 the oxygen was discontinued. During the next few days she was very weak and exhausted. Only occasional rales were heard in the lungs. The skin lesions were still discrete, but covered with a dark brown crust, which peeled away to reveal a pink and firm skin. Her most distressing symptoms were pain and salivation due to the ulcerations of the mouth and lips.

A blood transfusion was given on May 26, after which there was progressive improvement, with a gradual clearing of all skin and mouth lesions. There was marked desquamation of the skin of the trunk, hands and feet, with the loss of seven nails on the hands and feet. By May 31 her only symptoms were extreme weakness and superficial ulcerations of the lips. During this entire episode, from May 18 to May 31, the patient lost 22 lbs. in weight.

On June 3 the patient was restarted on barbiturate therapy without any toxic reactions. On June 1, a repeat EKG showed similar findings to that taken on May 6, except that there was now T wave inversion in all leads. This record also was interpreted as being compatible with hyperthyroidism. The patient continued to regain her strength, although she had an unexplained episode of temperature elevation from June 14 to June 17. A BMR taken on June 19 was plus 56.2.

The following day, June 20, the patient was started on propylthiouracil (2), with an initial dose of 100 mg. daily which was later increased to 150 mg. This medication was continued, as a preoperative measure, until August 10.

The patient reacted satisfactorily to this new regimen. She gradually regained her weight, reaching 169 lbs. by Aug. 20. However, her BMR fluctuated between plus 23 and plus 58 per cent. The last preoperative report was plus 40 per cent Aug. 16. Despite this high reading, her general condition was excellent, with complete regression of all symptoms and gain in weight as stated.

The EKG now showed some extensive changes. July 12, all the ST-T deviations had disappeared; there was only a T3 inversion, and the record was interpreted as a borderline curve. On Aug. 5 and Aug. 30 the records were similar to those of July 12.

Propylthiouracil therapy was discontinued on Aug. 10, as an immediate preoperative measure, in order to lessen the friability of the thyroid gland at operation. Obviously, the use of iodine at this time was impossible. Subtotal thyroidectomy was performed Aug. 20. The patient made an uneventful recovery, and was asymptomatic when discharged Aug. 31. Her BMR on this date was plus 13 per cent.

The patient returned to the clinic for post-operative observation Sept. 3. At this time patch tests were performed to determine her cutaneous iodine sensitivity. Three gauze patches, each one inch square, were placed at six inch intervals over the patient's back.

Patch #1 was impregnated with Lugol's solution.

Patch #2 contained one-half strength tincture of iodine, U.S.P.

Patch #3 was a saline control.

On the same date, three control subjects were tested, using the same technique. All tests were read in 24 hours. None of the control patients showed any evidence of skin sensitivity to the iodine solutions beyond the mere staining of the skin.

However, our patient complained of pain over two of the patch areas, when she returned the following day. Examination revealed that the skin under both the iodine patches had undergone marked reaction. These areas of skin were raised, discolored, acutely tender with multiple vesicles. Upon perforation of the larger vesicles, a thin yellow fluid escaped. This reaction was interpreted as positive evidence of skin sensitivity to iodine. (See Fig. 1.)



FIG. 1.—Patch test results with use of

- A. Saline
- B. A half strength tincture of iodine
- C. Lugol's solution

**Differential Diagnosis:** The only condition that was considered in the differential diagnosis, in this patient, was Stevens-Johnson's disease. This is characterized by the skin lesions of erythema multiforme bullosum, with involvement of the mucous membranes (1-4-5-6-8-9-10-11). The clinical story is that of a sudden onset of symptoms; fever of 100-102° F., weakness, ophthalmia, and a generalized skin eruption occurring within 36-72 hours from the onset of symptoms. The lesions first appear macular in type, and rapidly progress to the formation of large vesicles. The most classical lesions are those about the lips and mouth. Here are found large bullous lesions which erupt, and produce sloughs. Genital lesions which simulate those in the mouth and conjunctival surfaces have been described by Edgar and Sylvester (5), and Ageloff (1). The average time for the disappearance of the eruption is two to three weeks. The oral lesions heal more slowly, because of secondary infection. No specific etiological agent has been identified with this disease. It has been suggested that these are cases of sensitivity to Vincent's infection, but this has not been proven. There is no laboratory or clinical proce-

ture which is specifically diagnostic for this disease. In reviewing the literature, it was found that no drugs were administered immediately prior to the onset of symptoms, in any of these cases.

In the present case, the diagnosis of Steven-Johnson's disease was excluded for the following reasons:

- 1) The outbreak of these lesions occurred during the course of iodine therapy.
- 2) Iodine sensitivity in this patient was confirmed by patch tests.
- 3) The skin eruption occurred within 24 hours from the onset of symptoms. These lesions were far more numerous than those described in Stevens-Johnson's disease.

## DISCUSSION

Since Plummer's work in 1923, iodine has been used extensively in the management of toxic goiter. It is interesting to note that only two previous reports of iodism following the use of Lugol's solution are to be found in the literature. Barker and Wood (3), in 1940, reported seven cases of iodine toxicity in a series of 4,000 cases of hyperthyroidism which were treated at the Johns Hopkins Hospital. Guptill (7), in 1942, reported on an additional case of this type.

Of these eight cases, six were mild and all evidence of toxicity disappeared within one to two days after the drug was stopped. However, in two of these mild cases, a low-grade fever persisted for four to five weeks, although these individuals were asymptomatic. Two cases, both from the series of Barker and Wood, were severely toxic, and one of these terminated fatally. The other patient developed fever, conjunctivitis, enlarged lymph nodes, a generalized rash, and a definite jaundice. Her icterus index rose to 20, her liver was palpable, and tender. In this case the fever persisted for one week, the rash for two weeks, and the jaundice for one month. However, recovery was complete.

The fatal case was that of a Negro male, 56 years of age, who was started on Lugol's solution in a dose of 3 cc. daily. After two days of therapy, he developed a fever of 101.2° F. The dose of Lugol's was doubled in the hope that this might overcome the febrile response, but fever persisted and generalized pruritis developed. On the ninth day of treatment the drug was discontinued. Two days later, a saturated solution of potassium iodide was administered in its place, using 2 cc. the first day, and 3 cc. the second day. The patient responded with a fever of 104.2° F., a diffuse, reddish, macular eruption on the trunk, a hemorrhagic plaque on the palate, and coryza-like symptoms. The potassium iodide was discontinued but the eruption spread, the buccal mucosa became studded with petechiae, and the patient continued to be very sick.

Desquamating lesions of the tongue and palate appeared, and eight days after the potassium iodide was discontinued the patient became disoriented went into coma and died. Autopsy revealed that the mucosa of the mouth,

pharynx, larynx, trachea and bronchi were injected, and these mucous membranes were thinly coated with a grayish-white exudate. The liver, spleen, stomach and kidneys were hyperemic, with microscopically visible miliary, inflammatory, perivascular lesions.

Certain generalizations may be made on the basis of these nine cases of toxic response to Lugolization. The characteristic findings were as follows:

- 1) The first rise in temperature occurred between the second day and the eighteenth day after the start of Lugolization.
- 2) The maximum temperature ranged from 101° F. to 105° F.
- 3) A rash was present in six of the nine cases.
- 4) Five of these patients developed a conjunctivitis.
- 5) Seven of the patients had coryza-like symptoms.
- 6) Five of the patients had enlarged lymph nodes.
- 7) The WBC ranged from 5,000 to 12,200, but was above 10,000 in only 2 cases.
- 8) The original BMR determinations varied from plus 16 to plus 83 per cent.
- 9) Barbiturates were used in conjunction with Lugol's solution in all these cases. In most instances the barbiturates were continued or readministered after the Lugol's solution had been discontinued. None of these patients showed a further toxic response.

#### SUMMARY

1) A case of severe iodine toxicity following Lugolization is described. It was characterized by an abrupt onset, a fulminating course, high fever, diffuse and severe lesions of the skin and mucous membranes, coryza-like symptoms, enlarged lymph nodes, and only slight elevation of the W.B.C.

2) Following this episode, propylthiouracil was used in the further pre-operative preparation of this patient; with satisfactory results and absence of toxic reaction.

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# Letter to the Editor

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TO THE EDITOR:

## TREATMENT OF THIOURACIL AGRANULOCYTOSIS WITH STREPTOMYCIN

IN AGRANULOCYTOSIS the use of penicillin as a stop gap for infection, while the granulocytes are regenerating from their toxic effects, is well established. In complete agranulocytosis this regeneration usually begins within eight days. The thought occurred that streptomycin may be necessary in cases of this type which do not respond to penicillin. In the following patient with agranulocytic angina which followed the use of thiouracil, penicillin in large doses was not adequate and the patient still showed evidence of an infection on the sixth day of her illness. Gram negative organisms were found at this time on throat culture. Streptomycin<sup>1</sup> was substituted for penicillin. The patient recovered.

Agranulocytosis has numerous causes. The most common is a toxic reaction to some drug. At least twenty-one deaths from the use of thiouracil have been reported in the literature of agranulocytosis. One of the authors (B.S.) has observed five such cases, all of whom recovered. Two have been reported previously.<sup>2</sup> Since thiouracil has been such a frequent offender, propyl-thiouracil, a much less toxic drug, is now recommended in its place.

Harrison et al.<sup>3</sup> noted the disappearance of fever following the use of streptomycin for a period of eight days in a fatal case of agranulocytosis and aplastic anemia (amyloidemia<sup>4</sup>) following tridione. Streptomycin was discontinued and given again after a positive blood culture for *B. Coli* developed thirteen days later. Menorrhagia which was also present due to thrombocytopenia was treated by uterine packing which "contributed to the terminal infection and death."<sup>5</sup> Autopsy showed 96 per cent lymphocytes in the bone marrow. The following is a summary of the case history of one of our patients.

Mrs. E.S. was 30 years old and had one child. She was nervous, apprehensive and had slight exophthalmos. She had cramps and an urgent desire to defecate after each meal. Her teeth were in poor condition. The thyroid gland was diffusely enlarged to about twice the normal size. No bruit was noted. She had a marked tremor of the tongue and

<sup>1</sup> We are grateful to Dr. Charles S. Keefer and the Pfizer Company for release of the streptomycin used.

<sup>2</sup> Seligman, B. *Treatment of Agranulocytosis*. *J.A.M.A.* 129: 1123 (1945).

<sup>3</sup> Harrison, F. H., et al. *Fatality Following Tridione*. *J.A.M.A.* 132: 11 (1946).

<sup>4</sup> Seligman, B. *Amyloidemia vs. Agranulocytosis*. *J.A.M.A.* 130: 530 (1946).

<sup>5</sup> Dr. Harold Fink.

hands. Her weight was 103½ pounds, her pulse 140, her blood pressure 154/80 and her hemoglobin 75 per cent (Sahli). No signs of myocardial insufficiency other than tachycardia were noted. The basal metabolic rate was plus 44 per cent.

She was given 0.2 gram of thiouracil on June 25th. Thereafter, she was given 0.2 gram three times a day until August 4th when the drug was stopped because she devel-

E.S., 30

TREATMENT OF THIOURACIL AGRANULOCYTOSIS  
WITH STREPTOMYCIN

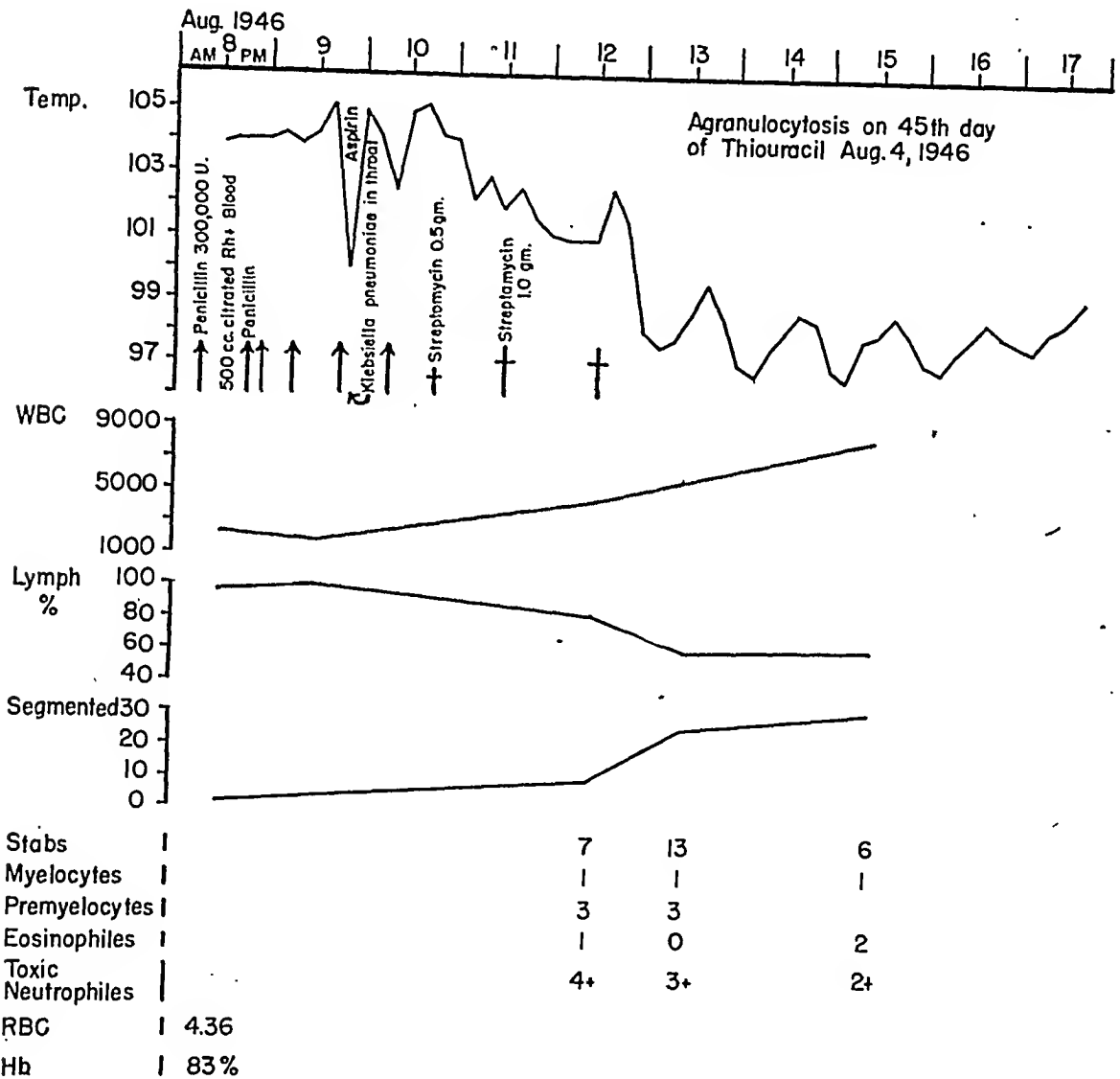


FIG. 1.

oped a sore throat. After one week of the thiouracil therapy she felt very much better, was less shaky, and slept and ate better. On July 26th her pulse was 84 and her weight 106 pounds. An interval blood count showed 6,200 white blood cells per cubic millimeter, 50 neutrophils (5 non-segmented), 44 lymphocytes and one monocyte. Her hemoglobin was 84 per cent and her red blood count was 4,333,000 per cubic millimeter. No bone marrow studies were performed.

On August 4th, 1946 the throat was diffusely red. The next day a blood count showed 3,500 white blood cells per cubic millimeter with no polymorphonuclear cells. The temperature was 104 degrees by rectum and she received 300,000 units of penicillin in oil that night. On August 6th the throat showed edema of the uvula, tonsillar pillars and palate. 300,000 units of penicillin in oil was continued twice daily. On August 7th the temperature was 105.2 degrees and the throat showed small, white, necrotic areas on the uvula and tonsillar pillars. The anterior cervical glands were enlarged and firm. On August 7th (see chart) she was admitted to a private hospital and penicillin was given in a dose of 300,000 units three times during the day. Her temperature varied between 103.8 and 104° F. She was given one indirect transfusion of 500 cc. of RH positive blood. The blood count showed 2,200 white blood cells per cubic millimeter. Of the 99 cells counted only two were non-segmented polymorphonuclear cells. On August 9th she had a small abrasion of the lip which was not inflamed. The throat was red with a small vesicle on the uvula and slight edema of the tonsillar areas. She had gingivitis. A systolic murmur was noted at the apex. The chest was clear. The abdomen was soft. The liver edge was palpable and tender just below the costal margin. Her temperature varied between 104.2° and 105° F. Following fifteen grains of aspirin in divided doses the temperature dropped to 100° only to rise again. The next day the temperature fluctuated between 102.6° and 105.2° F. At this time the white blood count showed only 1,850 lymphocytes per cubic millimeter.

A smear of the throat on August 9, 1946 revealed gram negative bacteria which, on culture, proved to be *Klebsiella pneumoniae* (5). She was given one gram of streptomycin daily in eight divided doses for two and a half days. The temperature fell after the drug had been started on August 10th at 3:30 p.m. The throat began to clear. A burning throat pain disappeared on August 11 when she began to look, feel and eat well. The liver edge was not felt. On August 13th the skin was cold and heat applied. The patient had remained well except for slight weakness.

She was given as adjuvants 10 cc. of pentnucleotide daily and 5 cc. of crude liver extract intramuscularly. With each injection of pentnucleotide she felt weak and sweated. During the acute phase of her illness the patient showed, as do other people with agranulocytosis, red, slightly elevated areas at the site of all the hypodermic injections. In this patient the areas were not noted after August 13 when a peripheral smear showed 41 per cent granulocytes. She was also given 10 minims of Lugol's solution three times a day, multiple vitamins and tablets of liver and iron orally. One and one-half grains of phenobarbital was given nightly. Her mouth was washed with dilute hydrogen peroxide. She was allowed out of bed on August 15th and discharged from the hospital August 17th. Penicillin had been stopped when streptomycin was started.

While agranulocytosis may occasionally disappear spontaneously, no one will deny the effectiveness of antibiotics in most cases. Penicillin is not considered completely effective if local lesions are not healed and the temperature reaction persists after 48 hours. Secondary invaders may have come to the fore, as in this case, after the growth of penicillin sensitive organisms has been arrested. We feel that streptomycin may then be indicated.

BERNARD SELIGMAN  
MORRIS WEINTROB  
Brooklyn, New York  
Nov. 17, 1946



## Association Notice

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The 29th annual meeting of the Association for the Study of Internal Secretions will be held Friday and Saturday, June 6th and 7th, 1947, in the Viking Room of Haddon Hall Hotel, Atlantic City, New Jersey, preceding the Centennial meeting of the American Medical Association.

For program see the February, 1947, issue of THIS JOURNAL.



# Announcement of Awards

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## ASSOCIATION FOR THE STUDY OF INTERNAL SECRETIONS

The Association for the Study of Internal Secretions has just announced that the award provided by E. R. Squibb and Sons has been conferred on Doctors Carl F. and Gerty T. Cori of Washington University, the award furnished by the Ciba Pharmaceutical Company has been made to Doctor Choh Hao Li of the University of California, and the newly established fellowship of Ayerst, McKenna and Harrison, Limited, has been given to Doctor Samuel Dvoskin of Columbia University.

The Squibb Award to Dr. and Mrs. Cori was based on major contributions which they have made on the action of adrenalin, insulin, and the anterior pituitary and adrenal cortical hormones concerned with metabolism. They described the physiology of glucose and lactic acid in the liver, blood and muscles under the influence of adrenalin and insulin. They have identified and purified the enzymes concerned in glycogen formation and have accomplished the synthesis of glycogen in vitro. Some of their recent studies have demonstrated that the first step in glucose utilization (the hexokinase reaction), a step common to both glycogen formation and the oxidation of glucose, is inhibited by the anterior pituitary body. The release of this pituitary inhibition by insulin has afforded the first demonstration of an action of insulin apart from living tissue. This revelation of the quantitative biochemical relation of hormones and enzymes opens a new era in endocrine research.

Dr. Carl F. Cori was born in 1896. He received the degree of Doctor of Medicine from the German University, Prague, Austria in 1920. He served as assistant in the medical clinics in Prague and Vienna and as assistant in Pharmacology at Graz. He was biochemist at the State Institute for the Study of Malignant Diseases, Buffalo, 1922-1930, and Assistant Professor of Physiology at Buffalo, 1930-1931. From 1931 to 1941 he was Professor of Pharmacology, and from 1941 to 1946, Professor of Pharmacology and Biochemistry at Washington University. Since 1946 he has been Professor and Chairman of the Department of Biochemistry at Washington University. Dr. Gerty T. Cori was born in 1896 and received the degree of Doctor of Medicine at Prague in 1920. She was assistant pathologist and biochemist at the State Institute for the Study of Malignant Diseases in Buffalo from 1922 to 1931. Since 1931 she has been Research Associate in Pharmacology and Biochemistry at Washington University.

The Ciba Award to Doctor Li was given for his significant contributions

in the separation and study of hormones of protein nature and the isolation of the adrenocorticotropic and growth hormones in homogeneous and highly purified states. Dr. Li was born in Canton, China on April 21, 1912. He received the degree of Bachelor of Science from Nanking University in 1933 and the degree of Doctor of Philosophy from the University of California in 1938. From 1933 to 1935 he was an instructor at Nanking University. Since that time he has served at the University of California as Research Associate, 1938-1942, as Assistant Professor, 1942-1946, and since 1946 as Associate Professor of Experimental Biology.

Doctor Samuel Dvoskin was named to receive the first Ayerst, McKenna and Harrison Fellowship. He received both the Ph.D. and the M.D. degrees from Columbia University in 1945 and in his studies on the testis and thyroid has shown unusual promise as an investigator. He will continue his work on the thyroid gland and thyroid hormone assays under the direction of Dr. Robert Loeb.



# Abstracts of

## CURRENT ENDOCRINE LITERATURE

Editor; D. A. MCGINTY. Collaborators: A. R. ABARBANEL, F. N. ANDREWS, B. L. BAKER, F. A. DE LA BALZE, ISRAEL BRAM, R. A. CLEGHORN, RUCKER CLEVELAND, C. D. DAVIS, ANNA FORBES, M. B. GORDON, H. S. GUTERMAN, M. M. HOFFMAN, R. G. HOSKINS, C. D. KOCHAKIAN, H. S. KUPPERMAN, H. L. MASON, JANET W. MCARTHUR, THOMAS H. MCGAVACK, A. E. MEYER, K. E. PASCHKIS, A. B. PINTO, J. R. REFORZOMEMBRIVES, E. C. REIFENSTEIN, JR., G. G. RUDOLPH, L. T. SAMUELS.

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### GENERAL

LIMARZI, L. R., C. L. PIRANI, AND R. J. KULASAVAGE. The effect of thiouracil on leucemia with a clinicopathologic report of a case of chronic myeloid leucemia that developed an extreme neutropenic leucopenia. *J. Lab. & Clin. Med.* 31(4): 470-471 (1946).

Four patients with myeloid leucemia and one with lymphatic leucemia were treated with thiouracil in daily doses varying from 0.2 to 3.0 gm. for periods varying from eight to ninety days. The drug was without effect upon the course of the disease or the clinical condition in any of these patients. In one patient with myeloid leucemia, the white blood count was reduced from 440,000 to 1,900 cells per cubic mm. with the complete disappearance of all granulocytic elements from the peripheral blood except basophilic polymorphonuclear cells which represented 33 per cent of the leucocytes present. In this patient at autopsy, "the bone marrow showed numerous myeloblasts and lymphocytes, and a complete aplasia of mature and immature types of neutrophils and eosinophils." Thyroid hyperplasia was not produced in any of the five patients studied.—*T.H.McG.*

NICHOLSON, W. M. Emotional factors in obesity. *Am. J. Med. Sci.* 211: 443 (1946).

The author compared the effect on weight loss of psychotherapy without the use of calculated diets (38 cases), calculated diets only (35 cases), amphetamine sulphate only (10 cases), and thyroid substance only (10 cases) in obese patients who were 10 kg or more over their calculated ideal body weight. Except for women in the menopause age group, all other patients with endocrine disease, inactivity due to debilitating illness or encephalitis were excluded from the series. Unless a patient had maintained a weight loss of 5 kg or more for 1 year, or if any relapses occurred after a year, the experiment was considered to be a failure. The results were considered to be successful in 26 patients who received only psychotherapy, and in 9 patients who received only diet; all others were considered to be failures. The author concludes that all obese patients studied have some type of psychoneurosis in varying degrees, and hence that psychotherapy and re-establishment of proper dietary habits is essential for permanent weight reduction.—*E.C.R., Jr.*

ROBERTS, J. G. Disappearance of secondary sarcomatous deposits in the lungs after stilboestrol therapy. *Brit. J.* 2: 693 (1946).

A case of endothelioma originating from the capsule of the shoulder joint, developed cachexia 18 months after fore-quarter amputation. X-ray examination showed multiple metastases in the lungs. Stilbestrol treatment was instituted, first by injection and later by mouth. She regained weight and lungs became radiologically normal. Patient is in excellent health 3 years and 9 months after amputation and 2 years, 1 month after beginning stilbestrol therapy.—*L.T.S.*

## GONADS

BLOCK, F. B. Ovarian Tumors. *Am. J. Med. Sci.* 212: 738 (1946).

The author reviews recent reports in the literature from a clinical point of view. The subjects treated include: granulosa-cell tumor, theca-cell tumors, arrhenoblastoma, dysgerminoma, Brenner tumor, adrenal rest tumor, teratoma, carcinoma, adenoacanthoma, Krukenberg's tumor, and fibroma.—*E.C.R., Jr.*

GREENHILL, M. H. A psychosomatic evaluation of the psychiatric and endocrinological factors in the menopause. *South. Med. Jour.* 39(10): 786-794 (1946).

In an investigation of the psychiatric and endocrinologic factors observed at the menopause, the author found that the psychiatric states could be associated with the menopause but were rarely a symptom of hypo-ovarianism. The evidence presented showed that the psychoneurosis never began with the menopause. The anxiety neurosis, hysteria, phobic states and compulsive-obsessive neurosis observed during the climacteric usually had been observed some time prior to the period of hypo-ovarianism. Twenty-five per cent of the patients who had psychoneurosis at the menopause had merely experienced a continuation of the symptoms which they had exhibited prior to that time. Involutional melancholia, tension states and reactive depressions were the most common psychiatric disorders observed at the climacteric. A comparison of the effectiveness of estrogens in the psychiatric patients and in those with the usual menopausal autonomic lability showed hormone therapy to be effective in only 3 per cent of the psychiatric patients as compared to alleviation of symptoms in 62 per cent of the women exhibiting autonomic lability. In concluding the author stated that there was no justification for the term 'menopausal syndrome' if it implied the presence of a psychiatric disorder in its definition.—*H.S.K.*

KARNAKY, K. J. Hydrogen ion concentration of the senile vaginal mucosa before and after estrogenic therapy. *South. Med. Jour.* 39(11): 906-907 (1946).

Determinations of the hydrogen ion concentration of the senile vagina were made before and after estrogenic therapy. The average pH of the normal vagina varied from 4.10 to 4.14, depending upon the area examined. The variation in the pH of the senile vaginal mucosa ranged from 6.27 to 6.42. Estrogenic substances lowered the pH of the senile vaginal mucous membrane. Stilbestrol, 0.5 mg. per day for 23 days, increased the hydrogen ion concentration more than ethinyl estradiol administered in daily doses of 0.09 mg.—*H.S.K.*

MASSON, G. The spermatogenic activity of  $\Delta^5$ -pregnenolone and of its esters. *Am. J. Med. Sci.* 212: 1 (1946).

Spermatogenic activity of steroid compounds is defined by the author as the ability of these substances to prevent testicular atrophy following hypophysectomy, or treatment with estradiol, small doses of testosterone or non-specific agents. Steroids are divided into 2 groups: those which are capable of restoring spermatogenesis in a testis previously rendered atrophic by hypophysectomy (compounds of the testosterone and androstenediol type), and those which do not possess this property (compounds of the progesterone type). The author has investigated the nature of the spermatogenic activity of  $\Delta^5$ -pregnenolone and of its acetate, propionate and benzoate on three groups of hypophysectomized rats, on two groups of rats given estradiol before or during pregnenolone treatment, and two groups of rats given estradiol before testosterone treatment. The results were evaluated in terms of the weight of the testes, the weight of the seminal vesicles, the weight of the prostate, the weight of the adrenals, and the evidence of spermatogenesis from histological examination. It was shown that testosterone accelerates, while pregnenolone inhibits the regeneration of the atrophic testis. On the basis of these results the author classifies testosterone as an *active* spermatogenic compound which directly stimulates the proliferation of the germinal epithelium, and pregnenolone as a *passive* spermatogenic agent, whose action consists merely in protecting and maintaining the testis in the condition in which it is at the commencement of treatment. The author states that this classification may serve as a basis for the rational selection of gonadotrophic steroids for clinical use. The steroids which belong to the testosterone and androstenediol type should be used only when the testis is already damaged, because, in the case of almost normal testes, there is danger of causing Leydig cell atrophy by such treatment, and this may represent a greater evil than the diminution in the spermatogenesis. Conversely pregnenolone should be used only if a comparatively normal testis is to be protected against subsequent damage. With this compound there is no reason to fear any unpleasant side-effects since at the daily dose of 10 mg. it is practically devoid of folliculoid, testoid, and corticoid activities and possesses only weak luteoid properties. The combination of testosterone and pregnenolone is suggested for the treatment of eunuchoidism without the danger of producing testis atrophy. —E.C.R., Jr.

TEILUM, GUNNAR. Gonocytoma. Homologous ovarian and testicular tumors. I. *Acta Path. et Microbiol. Scandinav.* 23(3): 242-251 (1946).

A detailed documentation is given of the previously demonstrated morphological congruity (Teilum, 1943) between the ovarian tumor misinterpreted by Schiller as mesonephroma and a most often adenopapilliferous, solid or cystic (sometimes teratoid), tumor of the testis. On the basis of the continuity demonstrated between this tumor form and, on the one side, seminoma (dysgerminoma), and on the other side, chorioma, the writer sets up two homologous tumor series in the ovary and testis, between which the present tumor is entered as an intermediate form.

Histogenetically, this entire group of tumors (gonocytomas) is characterized by their origin from early stages of the germ cells in the testis or from homologous remnants of the medullary cords in the ovary. In the ovary, thus not only the dysgerminoma but also the intermediate form (gonocytoma II) and the primary chorioma (gonocytoma III) appear as a morphogenetically defined group originating from a particular testicular

anlage. In connection with the critical evaluation of the term "mesonephroma ovarii," the description is given of a case of malignant ovarian tumor, which, in contrast to the true tumor from described by Schiller, has to be looked upon as a true mesonephroma.—*Author's Summary.*

TEILUM, GUNNAR. Arrhenoblastoma—Androblastoma. Homologous ovarian and testicular tumors. II. *Acta Path. et Microbiol. Scandinav.* 23(3): 251–264 (1946).

In connection with previous papers on homologous tumors of the ovary and testis, a case of feminizing testicular tumor is described showing complete morphological congruity with the virilizing arrhenoblastoma of the ovary described by Robert Meyer. The patient was a man, aged 53. The tumor had persisted for 30 years and had increased further during the last year. In the past 3 years there had been impotence and during the last year increasing gynecomastia. After the removal of the tumor, the gynecomastia began to subside. On the cut surface the tumor was intensely yellow in color. Histologically, a considerable lipid content was demonstrated. Thus the previously established testicular androblastoma (homologous with Meyers arrhenoblastoma of the ovary) is extended to include hormone-producing tumors showing all the differentiating stages known from the arrhenoblastoma. The hitherto unknown occurrence of this form of tumor demonstrates the correctness of Meyer's view concerning arrhenoblastoma of the ovary.

Further, a gradual transition is demonstrated from tumor tissue of the androblastoma type to clear-cut lipid cell tumor which shows morphological congruity with the virilizing lipid-containing ovarian tumors generally described as "luteoma," "adrenal tumors," "ovarian hypernephroma" and "folliculome lipidique." Accordingly, such ovarian tumors are now to be looked on as a particularly lipoidal form of ovarian arrhenoblastoma. In addition diffuse forms of tumors containing no lipid occur in the ovary as well as the testis which are to be considered as variants within the androblastoma series and may have a relative virilizing and feminizing effect. They are tumors with more or less pronounced differentiation in the direction of testicular interstitial-cell tumors and a homologous extraglandular, interstitial-cell tumor of the ovary.

The demonstrated homologous tumor series—respectively the seminoma (dysgerminoma) and androblastoma (arrhenoblastoma) series that may be encountered in the testis as well as the ovary in all forms of differentiation, are set up as the basis of a classification comprising both tumors of the testis and the "testicular" tumors of the ovary in keeping with the histogenetic and biological aspects. This further affords a far more clear-cut morphological differentiation of such ovarian tumors from other particular forms, e.g., the granulosa cell tumors.—*Author's Summary.*

WEAVER, J. D. Estrogenic hormones, often only a psychotherapeutic agent. *South. Med. Jour.* 39(7): 581–585 (1946).

The use of estrogens was decried and the reported beneficial effects usually observed after estrogenic therapy are considered merely on the basis of the psychotherapeutic action of the drug. The menopausal syndrome was described as a purely psychic reaction with its inception based upon the earlier conditioning of the patient. Data presented were slim, philosophical condemnation extensive, and abstinence from general use of estrogens was advocated. No indications for estrogens were discussed.—*H.S.K.*

## PANCREAS

BRIGGS, A. P. Some observations on severe diabetic ketosis treated with glucose and insulin. *J. Lab. & Clin. Med.* 31(11): 1244-1248 (1946).

Seven diabetic patients have been observed under treatment for severe ketosis. Quantitative estimations have been made of the free acetone and total acetone bodies of the blood and urine, and of the blood sugar. Qualitative determinations for the acetone bodies and sugar of the urine have been performed. The data obtained confirm the fact that the free acetone of the blood represents a substantial and reasonably constant (20 to 32 per cent) portion of the total acetone bodies of the blood. The total acetone bodies of the urine will not rise proportionately to those in the blood in cases with disturbances of renal function. The ferrie chloride test for diacetic acid in the urine is often negative, even in the presence of large amounts of ketone bodies. "Too much reliance should not be placed on tests for diacetic acid in the urine in the diagnosis of diabetic coma." The author favors the administration of glucose to the diabetic in coma "in quantities equivalent to at least half of the caloric requirements." He believes this is well justified therapy because (1) the patient in diabetic coma needs carbohydrate and (2) the administration of glucose lessens the chance of hypoglycemia. He recognizes the fact that the administration of glucose interferes with the use of blood and urine sugar levels as an index of the insulin requirement, but suggests that the tests for ketosis described in the paper may be satisfactorily substituted. In addition, the determination of plasma bicarbonate may be helpful, as suggested by others.—*T.H.McG.*

CONNOR, J. F., AND F. W. REYNOLDS. The two-dose dextrose tolerance test in the diagnosis of diabetes mellitus. *J. Lab. & Clin. Med.* 31(10): 1121-1128 (1946).

A statistical analysis has been made of the two-dose dextrose tolerance test performed on 26 normal persons, 82 with diabetes mellitus, 7 with renal glycosuria and 6 individuals in whom a disturbance of carbohydrate metabolism was questionable. The technique for carrying out the tests was similar to that employed by Rose and Exton except that all subjects irrespective of weight received 100 gm. of glucose as a 15 per cent solution divided into two equal portions given at the 0 and  $\frac{1}{2}$  hour periods respectively. Moreover, determination of the blood and urine sugar was made at the end of 2 hours in each individual. Of the 82 diabetic subjects, 22 required insulin; 49 were regulated satisfactorily without insulin; and 11 needed insulin in the early portion of their treatment but were later able to discontinue it. It is not clear from the text whether any of the tests were performed prior to regulation of the diabetes, but the assumption is that all of the subjects were reasonably well controlled when the study was begun. Of all the data obtained, the authors believe "the height of the one-hour blood sugar is the most reliable criterion for differentiating the normal person from the diabetic patient." They found that normal subjects gave readings at one hour of 150 mg. of glucose per 100 cc. or less, and that diabetic subjects showed 170 mg. or more of dextrose per 100 cc. of blood. The values for doubtful cases fell between these two figures. The two-hour reading was the next most reliable determination, and even if not done in all instances, should always be carried out in the doubtful case. At two hours, normal subjects had a blood sugar of 125 mg. or less per 100 cc. of blood, while values for the diabetic were 135 mg. or more per 100 cc. The one-half hour reading afforded little or no information, and from the viewpoint of the authors "may well be deleted from the procedure." A high



one-hour reading with a normal two-hour reading may be indicative of an excessively rapid absorption of dextrose; conversely, a normal one-hour figure with a high two-hour value may represent a delay in the emptying time of the stomach. As is the case with any other laboratory procedure, too much reliance should not be placed upon the results of the test alone. It should be interpreted in the light of all the clinical data.—*T.H.McG.*

DUMM, R. M., AND R. A. SHIPLEY. The simple estimation of blood ketones in diabetic acidosis. *J. Lab. & Clin. Med.* 31(10): 1162-1163 (1946).

An especially prepared nitroprusside powder has been used for the detection of acetone in body fluids. A single drop of the fluid to be tested is added to a pinch of the powder, 5 mm. in diameter, on white filter paper. When the reaction is positive a violet color reaction promptly occurs which is absorbed into the paper and may last for several hours. A drop of serum from blood containing a total of approximately 10 mg. of total acetone bodies per 100 cc. will give a positive reaction. Therefore, by a series of dilutions of the serum, it is possible to determine quickly the blood levels for acetone bodies within an accuracy of plus or minus 10 mg. per 100 cc. The authors have found their estimations with the method to check well with microchemical determinations within the range over which the method has been applied, viz. from 0 to 100 mg. of total acetone bodies per 100 cc. The method has been found particularly useful in (1) the routine management of diabetic coma; (2) the prompt recognition of surgical complications simulating coma in the diabetic as contrasted with coma itself and (3) the differentiation of minimal ketonuria due to minimal ketonemia from minimal ketonuria with depressed renal function and severe ketonemia.—*T.H.McG.*

ENGELHARDT, H. T., AND J. P. MELVIN, JR. The management of diabetes mellitus during pregnancy. *South. Med. Jour.* 39(9): 734-737 (1946).

Despite the fall in maternal mortality observed in diabetic pregnant women treated with insulin, infant mortality in these individuals has remained consistently high. Of 1490 diabetic women examined, 23 or 1.6 per cent, were obstetric cases. In this latter group, two patients were admitted to the hospital in coma, one of whom died, and three were seen in severe acidosis. There were only two cases of polyhydramnios, and a fetal mortality of 47 per cent was observed. The treatment of the pregnant diabetic and the physiologic changes in carbohydrate tolerance during pregnancy were classified according to the trimester the patient was examined. In the first trimester, the lowered renal threshold and decreased glucose tolerance resulted in increased insulin requirements. Little or no change was observed in carbohydrate tolerance or insulin requirements in the second trimester. The third trimester was characterized by sudden changes in carbohydrate tolerance. Therefore no dogmatic routine could be prescribed in this stage. The same dietary and weight restrictions observed in normal pregnant women should be ascribed to in pregnant diabetics. The authors advocated the use of the preconception dose of protamine zinc insulin and advised control of significant glycosuria by the use of crystalline insulin. All patients were delivered per vaginam. The authors did not attempt to explain the difference between the effect of insulin in reducing the maternal mortality rate and its failure to prevent fetal mortality over that seen in pregnant diabetics in the pre-insulin days.—*H.S.K.*

FISHER, A. E., AND HENRY BOLGER. Behavior and psychologic problems of young diabetic patients. *Arch. Int. Med.* 78: 711 (1946).

The problems which arose in 43 young diabetic patients from childhood through adolescence to maturity have been analyzed. Each patient had been observed for at least ten years. The type of home, its economic security and the contacts at school and in social life, all had especial influence on the reaction of the diabetic child to his disease. Specific problems of childhood became less disturbing with the onset of maturity. During adolescence, problems relating to vocation and marriage appeared. Maturity usually brought about improvement in behavior, with the desire for independence being its outstanding characteristic. Marriage for the young diabetic person was difficult, especially for the young women. However, most of the marriages have been happy ones. The specific effects of diabetes on behavior were unrelated to the age of onset, the duration or the severity of the disease. Diabetic regimentation, while necessary, at first produced behavior difficulties. The immediate and the more remote or cumulative effects of hypoglycemia on the brain were considered to be potentially serious. Psychopathic behavior in a serious form was exhibited in 3 patients; other minor behavior disturbances were frequent. Patients in the better economic group and with better home environment had higher intelligence quotients and made more satisfactory adjustments to diabetes.—*I.B.*

GABRILOVE, J. L. Chloride excretion during glycosuria in patients with diabetes. *J. Clin. Investigation* 25(2): 256-260 (1946).

Seven diabetic patients without ketosis were given diets containing from 2 to 4 grams of salt daily. While fully controlled from a diabetic viewpoint, the excretion of chloride on such a diet was averaged for a control period of seven days. Glycosuria was then induced by administering additional carbohydrate, by the withdrawal of insulin or by a combination of these procedures. There was no increase in the chloride excretion during glycosuria without ketosis even though the glycosuria was marked in degree and prolonged many days.—*T.H.McG.*

GOLDSTEIN, N. P., M. JACOBSON, I. R. TELFORD, AND J. F. ROE. Studies of pancreatic function. III. The effect of ligation of the pancreatic ducts upon the amylase and lipase content of the blood. *J. Lab. & Clin. Med.* 31(9): 999 (1946).

Values for the amylase and lipase of the blood serum were determined before and at periodic intervals for 8 months following the ligation of the pancreatic ducts of four cats. The usual immediate increase of these substances in the serum was observed postoperatively. At the one and two-month intervals all the animals showed values for amylase definitely below the control figures. Variations in the lipase content of the serum were less well marked, but a "recognizable decrease" could be observed 3 months postoperatively. From the third month onward, there was a gradual increase in the value for both substances, and by the end of the eighth month the serum concentrations for both were normal. In connection with these changes, it is of considerable interest to note that the pancreas of one animal was "completely atrophic"; that little acinar tissue was recognizable in two, while the fourth showed some "normal areas of acinar tissue."

Despite this variation in the histological picture, the behavior of serum amylase and lipase was quite similar in all four of the cats. The authors conclude that the subsequent return to normal of the values for serum amylase and lipase were due to extra-pancreatic factors. Moreover, the results of their experiments led them to believe that the determination of serum lipase is as nonspecific as the amylase test in the diagnosis of chronic pancreatic disease.—*T.H.McG*

KIMBLE, M. S., O. A. GERMEK, AND E. L. SEVRINGHAUS. Vitamin A and carotene metabolism in the diabetic as reflected by blood levels. *Am. J. Med. Sci.* 212: 574 (1946).

Plasma vitamin A and carotene levels were determined by dependable methods in 116 unselected diabetics. All possible types of deviation from the normal range in vitamin A: carotene relationship were observed. Values for one or both substances outside the normal range for the appropriate sex were found at hospital admission in the blood of 49% of the 59 males and 47% of the 57 female patients. The predominant type of deviation in the series as a whole was low vitamin A: low carotene. This was particularly noticeable among older patients and patients with infection. Least prominent of any trend was that toward high carotene with deficient vitamin A, an alteration that has been widely assumed to be characteristic of diabetes and that has been rationalized as resulting from a defect in the diabetic organism's ability to derive vitamin A from its pro-vitamin, carotene. Relatively uncommon in the series was the so-called diabetic carotenemia, considered without regard for the accompanying vitamin A level. From analysis of the individual cases in the light of present knowledge of the facts that can alter vitamin A supplies, availability, reserves, use, and the resultant blood levels, it is evident that there is no type of aberrant blood picture found in this series of diabetics that cannot be explained by considerations not necessarily peculiar to the diabetic, especially if general nutritional failure (less frequent than it used to be among diabetics) is included as a possible cause for inefficient use of ingested carotene.—*E.C.R., Jr.*

LANGNER, P. H., M. J. ROMANSKY, AND E. D. ROBIN. The fallacy of the Exton-Rose glucose tolerance test. *Am. J. Med. Sci.* 212: 466 (1946).

The authors contend that the Exton-Rose 2-dose, 1-hour, glucose tolerance test is based upon a physiologic misconception. They believe that in arriving at the conclusion that a second 50 gm. dose of glucose would have any appreciable effect upon the blood sugar curve in the short space of  $\frac{1}{2}$  hour, Exton and Rose failed to take into consideration certain physiologic facts: 1) the rate of absorption of glucose from the gastro-intestinal tract, 2) its rate of utilization, and 3) the natural shape of a glucose tolerance curve after giving 100 gm. of glucose, whether it is given in 1 dose or in divided doses of 50 gm., at  $\frac{1}{2}$  hour intervals. They cite evidence which indicates that the second dose of 50 gm. adds glucose at a time when there is still an excess in the upper intestinal tract. It would seem pointless to give a second dose of glucose at the height of the blood sugar curve, since in a normal individual the curve would fall whether a second dose were given or not given. In a diabetic the curve would continue to rise whether 1 initial dose or 2 doses at  $\frac{1}{2}$  hour intervals were given. Therefore, any difference in the effect which the administration of 1 dose of 100 gm. or 2 doses of 50 gm. each would have upon the blood sugar curve would not be manifested during the first hour or during the duration of the entire Exton-Rose test. The fact that the body requires 1 hour to dispose of 25 gm. of glucose given intravenously, thereby producing the maximum stimulus, makes it seem

unlikely that the disposal of an oral dose of 50 gm. could occur in  $\frac{1}{2}$  hour and any appreciable response be manifested to a second dose of 50 gm. The results of a series of well-controlled glucose tolerance tests are presented to support the contention that after giving 100 gm. of glucose in 1 initial dose the blood glucose curve is essentially the same as that obtained after giving 100 gm. in 2 divided doses of 50 gm. initially and 50 gm. at  $\frac{1}{2}$  hour (the Exton-Rose procedure). They point out that the interruption of the oral glucose tolerance test at 1 hour and the consequent use of the peak value as the criterion is much less accurate than the use of the 2 or 3 hour value of the glucose tolerance curve. They conclude that the Exton-Rose test is based on an hypothesis that is physiologically unsound, that the test is not a physiological entity, and that it is not basically different from the 1-dose glucose tolerance test.—*E.C.R., Jr.*

LAZAROW, A., AND S. L. PALAY. The production and course of alloxan diabetes in the rat. *J. Lab. & Clin. Med.* 31(9): 1004-1015 (1946).

The purpose of this work was to develop a method by which diabetes could be consistently produced in the rat. In all, 77 rats were injected with alloxan either intraperitoneally or intravenously. The variability of results obtained by intraperitoneal administration was ascribed to the "highly variable factor of destruction," which is minimized when the drug is given rapidly by the intravenous route. An injection of 40 mg. per kg. intravenously produced about the same degree of diabetes as a dose of 200 mg. per kg. intraperitoneally, and did so much more consistently. Dosages of from 50 to 200 mg. per kg. intraperitoneally and of from 15 to 100 mg. per kg. intravenously were tried. At the highest dosage level used intraperitoneally, 75 per cent of the animals developed diabetes, but appreciable renal damage was invariably present. Following the intravenous injection of alloxan, there was an initial hyperglycemia lasting three hours or more, followed by a very short period of hypoglycemia and finally a permanent hyperglycemic phase, usually reached by the end of 48 hours. All animals responded in the above fashion when 40 or more mg. per kg. were given intravenously, whereas only 75 per cent responded similarly to the largest doses (200 mg./kg.) intraperitoneally. The severity of the blood sugar responses varied directly with the size of the dose of alloxan. Renal damage was not significant in animals receiving intravenously a dose of 40 mg. or less per kg. This is in contrast to the fact that azotemia and renal damage were invariably present when the same degree and severity of diabetes was caused by the intraperitoneal injection of 200 mg. per kg. As a rule, by the use of a single intravenous dose of 40 mg. per kg. of body weight, it was possible to develop a moderately severe to severe diabetes in which the values for blood sugar continued to rise gradually to attain a height of from 400 to 600 mg. per 100 cc. within several weeks. The authors believe that the intravenous use of alloxan affords a dependable way to produce experimental diabetes consistently, and feel that the administration of a dose of 40 mg. per kg. of body weight will be followed by damage to the pancreas resulting in the loss of function of the beta cells without serious lesions in the liver or kidney.—*T.H.McG.*

PAGE, R. C., AND E. H. LANG. Study of absorption from crystalline insulin pellets and solutions at various sites in rabbits. *J. Lab. & Clin. Med.* 31(10): 1144-1147 (1946).

Hand-compressed pellets of crystalline zinc insulin varying in size from 2.9 to 6.5 mg. with a potency of 25.89 units per milligram were implanted subcutaneously, intramuscularly, or intrasplenically into rabbits. Blood sugar values were determined just

prior to the implantation and at 4, 5, 24 and 30 hours thereafter. All control animals survived the 30 hour period of observation. Eighty per cent of the animals with subcutaneous implants died of insulin shock within 24 hours, whereas 40 per cent of those with the intramuscular and intrasplenic implants, respectively, had succumbed in a similar manner within the same period of time. At 30 hours, the surviving animals of the subcutaneously implanted group showed normal values for glucose in the blood. For the subcutaneous and intramuscular routes of implantation, the most pronounced hypoglycemic effect was observed between the 4th and the 5th hours and had disappeared in the surviving animals by the end of the 24 hour period. In the case of the intrasplenic implantations, the insulin effect was much more prolonged with an average value for blood glucose at 24 hours in the 6 surviving animals of 51 mg. per 100 cc. The more rapid and intense hypoglycemia produced by the subcutaneous implantation was attributed by the authors to mechanical factors causing a more rapid breakdown of the pellets. This conclusion seemed all the more valid in view of the fact that no significant differences in the action of insulin in rabbits between intramuscular and subcutaneous injections of watery solutions could be demonstrated with regard to the speed of onset and depth of hypoglycemia. Despite the fact that the absorption of insulin from the splenic implants closely followed the route normally taken by endogenous insulin after its release from the pancreas, the authors could detect "no appreciable hepatic regulation."—*T.H.McG.*

REVENO, W. S. Thiouracil effect in diabetes mellitus complicated by hyperthyroidism. *Am. J. Med. Sci.* 211: 174 (1946).

According to the author, diabetes mellitus and hyperthyroidism occur together in two different combinations: (1) secondary hyperthyroidism (toxic adenoma) which becomes manifest after the diabetes mellitus has appeared, and 2) primary hyperthyroidism (exophthalmic or toxic diffuse goiter) which antedates the diabetes mellitus in appearance. The author treated 6 patients with this combination of disorders with thiouracil and obtained improved control of the diabetes in the 4 of the secondary type; the other two patients failed to respond favorably or became worse. Of the 13 patients previously reported in the literature the 4 that responded favorably were the only cases of the secondary type. The author believes that thiouracil is as effective as thyroidectomy in diabetics who have secondary hyperthyroidism, but that in those who have primary hyperthyroidism or exophthalmic goiter thiouracil exerts little favorable influence on the control of the diabetes although it does control the manifestations of thyrotoxicosis.—*E.C.R., Jr.*



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## RADIO IODINE: ITS USE AS A TOOL IN THE STUDY OF THYROID PHYSIOLOGY\*

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WITH the discovery of artificial radioactivity by Joliot and Curie (1), the building of the cyclotron by Lawrence and his associates (2, 3), and the development of the uranium chain reacting pile (4), the physicists have offered to biologists and physicians valuable tools for the study of intermediary metabolism. Indeed, the discovery of radioactive tracer techniques has been likened in importance (5, 6) to the invention of the microscope. The particular advantage of studies with these techniques lies in their applicability to the investigation of metabolic processes in physiologic equilibrium.

As Marine (7, 8, 9, 10) and his associates demonstrated in 1915 and 1916, the thyroid gland is unique in that it has the ability to collect iodine selectively in relatively large quantities. It is, therefore, not surprising that studies with the radioactive isotopes of iodine have proved readily applicable and illuminating in the study of thyroid physiology.

In 1937, a cooperative investigation for the study of thyroid physiology with radioactive isotopes of iodine was started in the Physics Department of the Massachusetts Institute of Technology and the Thyroid Clinic of the Massachusetts General Hospital. Very soon thereafter a similar project was undertaken at the University of California. Since then investigators in

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institutions throughout the scientific world have used similar techniques for the study of problems related to thyroid physiology. In the laboratory these studies have been done in experimental animals and with in vitro techniques on slices of thyroid tissue. In the clinic radio iodine tracer methods have been applied in studies of normal human beings and of patients suffering from various thyroid maladies.

Five isotopes of radioactive iodine have been described in detail by Livingood and Seaborg (11). They are isotopes produced by bombardment of iodine, tellurium or antimony by neutrons, deuterons or helium ions respectively. The first available radioactive iodine was prepared by the action of slow neutrons on iodine. It has also been prepared by deuteron bombardment of tellurium. Its atomic weight is 128. Its half life of 25 minutes put a limit on the time of observation in the early studies with radioactive iodine. Bombardment of iodine with fast neutrons gives rise to a radioactive element which has a half life of 13 days and has an atomic weight of 126. Chemically, it is identifiable as iodine and therefore, is known as  $I^{126}$ . This same isotope has also been prepared by bombarding antimony with helium ions. Another radioactive isotope of iodine arises from the bombardment of antimony with helium ions. Its half life is four days and its atomic weight is 124. The bombardment of metallic tellurium with deuterons gives rise to two radioactive isotopes of iodine. One has an atomic weight of 130 and a 12.5 hour half life. The other has an atomic weight of 131 and a half life of 8 days. The eight day half life of this last named isotope makes it a most convenient preparation for use in biological experimentation. In the literature these various isotopes are identified by their atomic weights or by designating their half life periods.

### **The Collection of Iodine by the Thyroid and the Effect of the Thyrotropic Hormone.**

Hertz and his associates (12, 13, 14, 15) have demonstrated that the normal thyroid collects far greater amounts of administered labelled iodine than do other tissues. They have also observed that thyroids made hyperplastic by the administration of thyrotropic hormone or certain nitriles or by cabbage feeding have a greater affinity for iodine than do normal thyroids. The thyroids of animals previously treated with iodine collect smaller amounts of the administered tracer iodine than do the thyroids of normal animals.

LeBlond and Sue (16, 17, 18) have also demonstrated that thyroid tissue collects selectively more iodine than do other tissues of the body. They have observed that the thyroids of hypophysectomized animals collect much less iodine than do the thyroids of intact animals, but much more than do other tissues of the body. The thyroids of animals previously

treated with thyrotropic hormone collect much more iodine than do the thyroids of normal control animals.

LeBlond (19) has used radioactive iodine in studying the anatomic distribution of iodine in the rat thyroid. The thyroids were removed and sectioned after radioactive iodine had been administered. Radioautographs were made by placing the sectioned thyroids against photographic film. The sections when stained were compared with the shadows produced by emanations from the deposited radioactive iodine. With these studies it has been observed that iodine is deposited primarily in the colloid. In another study with autoradiograms (20), LeBlond has observed a predominance of basophilic follicles in the stimulated thyroids. Yet, he has found that these follicles always contain less of the radioactive iodine than do the acidophilic follicles. He interprets these results as indicating that the turnover of iodine proceeds more rapidly in the basophilic follicles than in the acidophilic follicles.

Ariell and his associates (21), have demonstrated that though the thyroid collects by far the highest percentage of the administered iodine, lung and kidney tissues collect relatively more than do other tissues of the body. They could not demonstrate any radioactivity in the expired air of their animals.

LeBlond and Mann (22) have observed that rats on an iodine deficient diet, or receiving an iodine deficient diet supplemented by soy beans, collect more iodine in their thyroids than do the control animals. The soy bean fed animals have a greater affinity for the administered iodine than do animals on an ordinary iodine deficient diet.

Keating (23) has used radioactive iodine in a collaborative study with the Radioactivity Center of the Massachusetts Institute of Technology and with members of the Thyroid Clinic of the Massachusetts General Hospital. The objective of this study was to determine whether any relationship could be demonstrated between the anatomic changes effected by the injection of thyrotropic hormone and changes in the thyroid's capacity to collect iodine or to release previously stored iodine. The day old male chick was used as the test animal. In each experiment of this study the animals were divided into two sub-groups. One group served for the study of iodine collection, and the other group was used to determine the effect of treatment on the thyroid weight and on the mean acinar cell height done according to the techniques described by Rawson and Starr (24) and Rawson and Salter (25). The animals were injected with 2 gamma of iodine as sodium iodide labelled with radioactive iodine with a half life of eight days ( $I^{131}$ ) twenty-four hours after the last injection of the thyrotropic hormone. Four hours later the animals were sacrificed and their thyroids removed. The percentage of labelled iodine collected was de-



terminated by comparing the radioactivity in an alkaline digest of the thyroids with that in a known amount of the original preparation of radioactive iodine.

In the first experiment 120 chicks were divided into five groups, and the effects of varying doses of thyrotropic hormone were determined. Thyrotropic hormone in doses of  $\frac{1}{8}$ ,  $\frac{1}{4}$ ,  $\frac{1}{2}$ , and 1 unit was administered by subcutaneous injection for 4 successive days (26). It was observed that with increasing doses of thyroid stimulating hormone both the weights of the thyroid glands and the mean acinar cell heights continue to increase

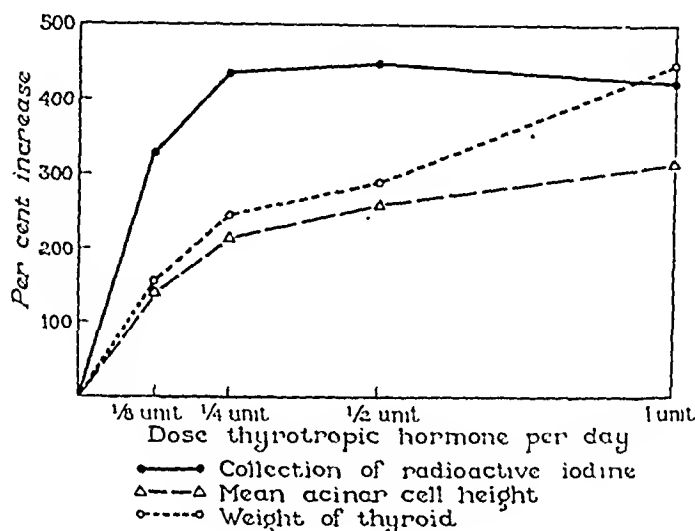


FIG. 1. The effect of varying doses of thyrotropic hormone on the collection of radioactive iodine, mean acinar cell height and on the weight of the thyroids of baby chicks. Each point on the figure represents the average per cent increase over the average observed in untreated controls.

though the latter tend to level off with larger doses. The capacity of the thyroid to collect the labelled iodine apparently reaches a plateau when the daily dose of thyrotropic hormone exceeds  $\frac{1}{4}$  of a unit. The average per cent increase over the normal in mean cell height, thyroid weight and iodine collection for each dose group is illustrated in Figure 1.

In the second experiment the effects of repeated doses of thyrotropic hormone were determined on 278 chicks. The treated animals were injected with one unit of thyrotropic hormone daily. Beginning with the first dose animals from the treated and control groups were sacrificed twenty-four hours after each injection. It was observed that the mean acinar cell heights increase rapidly following the initial dose and increase by successively smaller increments following each subsequent dose. The weights of the thyroids increase slowly at first but later increase precipitously. By the fifth day the increase in weight far exceeds the increase in cell height.

Figure 2 illustrates the average increase over the normal in each of these responses as the number of injections with thyrotropic hormone is increased.

It would appear that the anatomic changes produced by administering thyrotropic hormone are, first, an increase in the size of the existing thy-

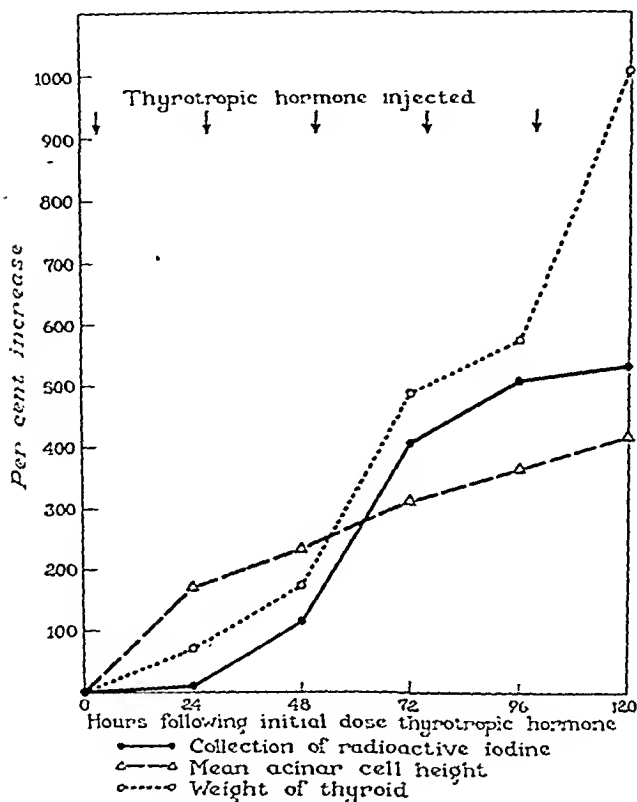


FIG. 2. The effect of daily injections of thyrotropic hormone on the collection of radioactive iodine, mean acinar cell height and weight of the thyroid of baby chicks. One unit of thyrotropic hormone was given daily. One group of chicks was sacrificed 24 hours after each injection. Each point on the figure represents the average per cent increase over the average observed in the untreated controls.

roid cells and, second, a multiplication of cells after the cellular hypertrophy has reached a ceiling. The latter change would account for much of the increase in weight, though some of this weight increase is no doubt due to an increase in vascularity. There is no change in capacity to collect radioactive iodine in the first twenty-four hours. The increase in this capacity is impressive between 48 and 72 hours, after which there is no further increment even though the thyroid mass continues to increase. This same

phenomenon of decelerating increase in the thyroid's avidity for iodine with greater thyroid stimulation has also been observed by LeBlond and Sue. It would seem that rapidly growing and hyperplastic thyroid tissue exhibits a lesser avidity for iodine than does tissue which shows only hypertrophy. This decrease in function may be attributable to the fact that hyperplastic cells are too young to be capable of collecting iodine. The work of Gorbman and Evans (27) would tend to substantiate such an explanation. They demonstrated that foetal rat thyroids are totally incapable of

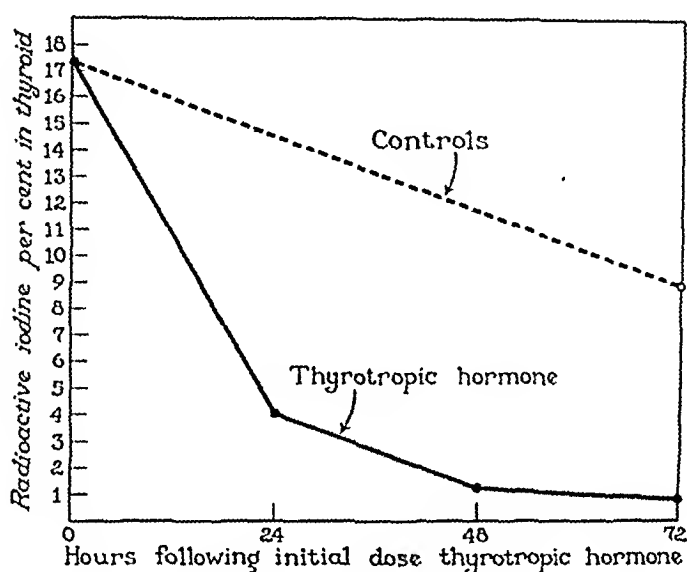


FIG. 3. The effect of daily injections of thyrotropic hormone on the loss of radioactive iodine from the thyroids of baby chicks. The radioactive iodine was given to all of the chicks before any food or water had been administered. One unit of thyrotropic hormone was administered daily for three days and one group of treated chicks was sacrificed 24 hours after each injection of thyrotropic hormone. Controls were sacrificed at the beginning and at the end of the experiment. Each point on the figure represents an average per cent of the administered radioactive iodine demonstrated in the thyroids of each group.

collecting iodine until the nineteenth day. Even then the capacity for collecting iodine is inferior to that observed on the twenty-first day. It is also possible that this apparent decrease in function of the markedly stimulated thyroid is an artifact. The excretion of iodine by the hyperplastic thyroid may be so rapid that the rate of iodine absorption cannot be determined accurately by the methods used in this study. Evidence in support of this explanation may be found in the next experiment (23) in which the effect of the thyrotropic hormone on the release of stored iodine was studied.

Fifty starved chicks, 48 hours old, were given 0.5 micrograms of iodine

labelled with radioactive iodine. The treated animals received one unit daily of thyroid stimulating hormone for three days. One group was sacrificed twenty-four hours after each injection. Controls were sacrificed on the first and third days. The loss of stored iodine was greatly increased by each injection of thyroid stimulating hormone. There was a loss of 76 per cent of the iodine in the first twenty-four hour period. At the end of three days the iodine loss in the treated animals amounted to 96 per cent as compared to a loss of 48 per cent in the untreated controls. The loss of stored iodine by the thyroid in response to the administration of thyrotropic hormone is illustrated in Figure 3.

The studies of Perlman, Morton and Chaikoff (28, 29) also indicate that treatment with the thyroid stimulating hormone causes a rapid release of hormonal iodine. They have observed that the plasma of guinea pigs treated with thyroid stimulating hormone contains a greater percentage of organically bound radioactive iodine than does the plasma of untreated animals. This phenomenon appears to be reflected in the tissues. Twenty-six hours after the injection of thyrotropic hormone almost all of the radioactive iodine contained in the muscle, liver and intestine is found in the thyroxine-like fraction. In the untreated animals more than half of the initially labelled iodine in the same tissues is in the inorganic form.

#### Studies on the Chemical Transformation of Administered Radioactive Iodine.

Perlman, Morton and Chaikoff (28, 29, 30, 31) employing tracer amounts of radioactive iodine without carrier have executed a series of studies on the distribution of various fractions of thyroid iodine under different experimental conditions. They have reported that as the time interval after administering tracer iodine increases, there is a concomitant increase in the proportion of labelled iodine in the thyroxine-like fraction. These same investigators with Anderson (32) have found that, though the thyroids of hypophysectomized rats collect much less of the tracer iodine than do the thyroids of intact animals, the amount collected is real and measurable. The iodine is readily converted into the diiodotyrosine-like fraction but very little of the tracer is demonstrable in the thyroxine-like fraction.

Mann, LeBlond and Warren (33, 34) have studied the conversion of radioactive inorganic iodine to the diiodotyrosine and thyroxine-like fractions. At 2½, 28 and 48 hour intervals the greatest concentration of the labelled iodine is demonstrable in the diiodotyrosine-like fraction. The longer the time interval the greater is the amount of tracer iodine found in the thyroxine-like fraction.

Morton and associates (35) have studied the formation of thyroxine and

diiodotyrosine by the completely thyroidectomized animal. They have reported that totally thyroidectomized rats are able to convert iodide to diiodotyrosine and thyroxine. (The completeness of the thyroidectomy was confirmed by histologic examination of serial sections from the base of the tongue to the heart and by radioautographic demonstration of the absence of iodine-concentrating tissues in the neck and mediastinum.) As early as 96 hours after its injection, 30 per cent of the radio iodine demonstrable in the liver and in the small intestine is organically bound; 20 per cent is found in the diiodotyrosine-like fraction and 8 per cent in the thyroxine-like fraction. The presence of newly formed radio-diiodotyrosine and radio-thyroxine was established in the thyroidectomized rat by demonstrating a constant radioactivity per unit of crystalline material obtained during several recrystallizations of (1) a mixture of the thyroxine-like fraction of the tissue and inert crystalline thyroxine and (2) a mixture of the diiodotyrosine-like fraction of the tissues and inert crystalline diiodotyrosine.

These observations on the chemical transformation of radioactive iodine are very interesting and may be of fundamental importance. However, it must be remembered that the possibility of an exchange taking place between the natural occurring iodine and the newly administered labelled iodine is real. There is even greater danger of an exchange occurring between the radioactive iodine and added crystalline thyroxine or diiodotyrosine. Miller and co-workers (36) using radioactive iodine as iodide have observed an exchange between the iodide ions and the iodine of diiodotyrosine at a pH of 4 to 5.5. These results do not signify that exchange reactions necessarily occur *in vivo* among various iodine compounds. However, they do indicate the need for caution in interpreting the results of studies with radioactive iodine on the synthesis and metabolism of the thyroid hormone.

Lein (37) in his studies with radioactive iodine has observed that as inorganic iodine is converted to an organic fraction more total iodine is demonstrable in the gland.

LeBlond, Gross, Peacock, and Evans (38) have observed that rats exposed to cold at the time of maximal stimulation fix 2.7 times as much radioactive iodine in their thyroids as do the control rats. Separation of the iodine fractions of the thyroids at various time intervals indicates that the turnover of thyroxine and the excretion of iodized products proceeds at double the normal rate in animals exposed to cold.

### **The Effect of Certain Thyroid Inhibitors on the Metabolism of Iodine.**

Chaikoff (39, 40) and his associates have utilized radioactive iodine for the study of iodine metabolism with *in vitro* techniques. They have

incubated thyroid slices in Ringer's solution containing tracer amounts of radioactive iodine and then determined the fractions of inorganic, diiodotyrosine-like and thyroxine-like radioactive iodine in the thyroid tissue. After three hours of incubation as much as 12 per cent of the added iodine in the thyroid slices is in the thyroxine-like fraction and as much as 70 per cent is in the diiodotyrosine-like fraction when slices of rat thyroids are studied. Slices of thyroids from dogs and from sheep convert less of the iodine into the organic fractions. In another study (41) they have reported that cyanide, azide, sulfide and carbon monoxide inhibit the formation of diiodotyrosine and thyroxine. Decreasing the oxygen concentration also inhibits the conversion of inorganic to organic thyroid iodine. They conclude that the formation of diiodotyrosine and thyroxine is linked with aerobic oxidation in which the cytochrome and cytochrome oxidase systems are involved. Cyanide and sulfide inhibit the accumulation of labelled iodine by the thyroid slices, whereas azide has no effect on the collection of iodine. By similar techniques (42) they have demonstrated that various sulfonamides inhibit the formation of diiodotyrosine and thyroxine without affecting the iodine concentrating power of the thyroid slices. In another *in vitro* study (43) they have observed that the addition of a small excess of iodide to each flask of medium inhibits the conversion of inorganic radioactive iodine to diiodotyrosine-like and thyroxine-like iodine.

With the same *in vitro* techniques this group of investigators (44) has studied the effect of various goitrogenic substances on the conversion of iodine to diiodotyrosine and thyroxine by slices of sheep thyroids. They have observed that thiourea, thiouracil, allylthiourea, and para amino benzoic acid strongly inhibit the formation of diiodotyrosine and thyroxine but do not prevent the collection of inorganic iodine. Methyl cyanide has no effect on the collection of iodine nor on the conversion of inorganic iodine to the organic fractions. Potassium thiocyanate not only depresses the conversion of inorganic iodine to diiodotyrosine and thyroxine but also prevents the collection of iodine by thyroid slices.

This group of investigators (45) has also studied the influence of thiouracil on the formation of thyroxine and diiodotyrosine by the intact thyroid gland of the rat. The feeding of thiouracil for seven days depresses the uptake of radioactive iodine. The thyroids obtained from rats injected with radio iodine on the last day of thiouracil feeding as well as those removed from rats injected seven days after its feeding had been stopped contained about half the amounts of radio-thyroxine and about one third the amounts of radio-diiodotyrosine found in the normal glands. Recovery, as judged by the iodine concentrating capacity of the thyroid and the amounts of radiothyroxine and radio-diiodotyrosine formed is complete two weeks after the administration of thiouracil has been discontinued.

We have used the radio iodine tracer techniques in studying the mode of action of certain goitrogenic agents on the thyroids of experimental animals and of patients who developed goiters while taking these agents therapeutically.

Since Astwood (46) had shown that the goitrogenic action of potassium thiocyanate can be prevented by iodine and that thiouracil is an iodine resistant goitrogen we (47) undertook to determine whether any difference could be demonstrated in the collection of radioactive iodine by thyroids of animals made goitrous with these two agents. Seventy-five rats were maintained on a Remington borderline iodine deficient diet. The daily intake of iodine in these animals was estimated to be 0.6 gamma. One group of twenty-five rats received thiouracil in the drinking water in a concentration of 0.1 per cent. A second group of twenty-five animals received a 0.25 per cent solution of potassium thiocyanate as drinking water. The remaining 25 animals served as controls. After the experimental animals had received the drugs for a twenty-eight day period, they all received by intraperitoneal injection two gamma of iodine as sodium iodide labelled with radioactive iodine. The animals were sacrificed four hours later. Their thyroids were removed and the radioactivity of an alkaline digest of each thyroid was compared with that of a standard equal to the amount of the injected labelled iodine. The average thyroid weights per 100 Gm. of body weight of the control animals was 10 mg.; of the potassium thiocyanate treated animals 23.0 mg.; and of the thiouracil treated animals 32.0 mg. The average collection of labelled iodine by the thyroids of control animals was 56 per cent; of the potassium thiocyanate treated animals 86 per cent; and of the thiouracil treated animals 10 per cent. The average thyroid weight and per cent collection of iodine by each of these groups is illustrated in Figure 4.

Similar observations have been made on two patients who developed goiters and myxedema while taking these same drugs. The first patient (48) was a hypertensive who developed a large vascular goiter and myxedema after receiving potassium thiocyanate for one year. The second patient was an acromegalic whose growth Drs. F. Albright and E. Reifensstein attempted to decrease by inducing hypothyroidism with thiouracil. Six months after the treatment had started he developed myxedema and a very large vascular goiter. Biopsy specimens were obtained from the goiters of both of these patients. Histologically both were wildly hyperplastic. (See Figures 5 and 6.) Though these goiters were similar in histologic appearance, they differed radically in their capacity to handle radioactive iodine. The thyroid of the patient treated with potassium thiocyanate retained 76 per cent of a tracer dose of radioactive iodine; that of the thiouracil treated patient retained none of it. (See Figure 7.)

It appears that thiouracil blocks the uptake of iodine and thus prevents the formation of an iodinated hormone. The result is a fall in the rate of metabolism and hyperplasia of the thyroid. It has been suggested that the latter is due to increased pituitary thyrotropic activity. Larson, Keating, Peacock and Rawson (49, 50) have demonstrated that thiouracil promptly produces a block to the collection of iodine. They treated 60 chicks with

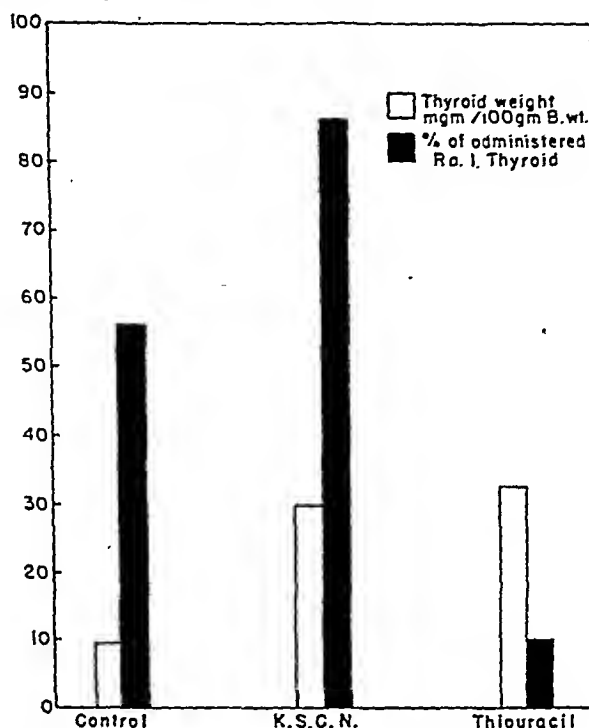


FIG. 4. The collection of radioactive iodine by the thyroids of rats made goitrous by the administration of potassium thiocyanate and thiouracil. The white columns represent the average thyroid weights (mg.100 Gm. body weight). The black columns represent the average per cent collection of administered radioactive iodine by the thyroids.

one unit of thyrotropic hormone daily for four days. On the fifth day the experiment was begun. Forty chicks received 10 mg. of thiouracil by injection at the beginning of the experiment. Groups of 10 animals each received radioactive iodine 1, 6, 12, and 24 hours after the thiouracil had been administered. Ten control animals received radioactive iodine at the beginning of the experiment and were sacrificed four hours later. The remaining 10 control animals received radioactive iodine 24 hours later. The control animals collected 27 per cent of the administered iodine in their thyroids whereas the animals which had received the iodine one hour





FIG. 5. Section of thyroid biopsy specimen removed from patient who developed a goiter while taking potassium thiocyanate. ( $\times 100$ )



FIG. 6. Section of thyroid biopsy specimen removed from patient who developed a goiter while taking thiouracil. ( $\times 100$ )

after thiouracil treatment collected only 0.7 per cent of the administered iodine. Those animals which had received the radio iodine 12 hours after the thiouracil was administered showed some escape from the thiouracil block to the collection of iodine. Twenty-four hours after the administration of thiouracil, the escape was almost complete. These animals collected

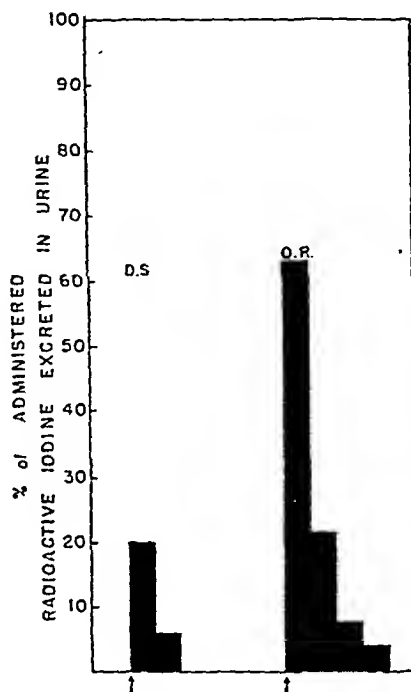


FIG. 7. The excretion in the urine of administered radioactive iodine by patients having struma mediceamentosa. The column on left represents the per cent of administered radioactive iodine excreted by a patient who developed a goiter while taking potassium thioeyanate. The column on the right represents the per cent of administered radioactive iodine excreted by a patient who developed a goiter while taking thiouracil

23 per cent of the administered iodine, controls sacrificed at the same time collected 29 per cent of the administered iodine. (Figure 8). The minimal dose of thiouracil necessary to produce this block was found to be 5 mg. (Figure 9).

McGinty and Rawson (51) have determined the minimal dose of various goitrogenic agents necessary to produce a 90 per cent or greater block to the collection of radioactive iodine by the thyroids of rats and chicks. Rats which had been maintained on an iodine deficient diet for a period of weeks simultaneously received varying doses of each drug and then were given a tracer dose of radioactive iodine one hour after administration of

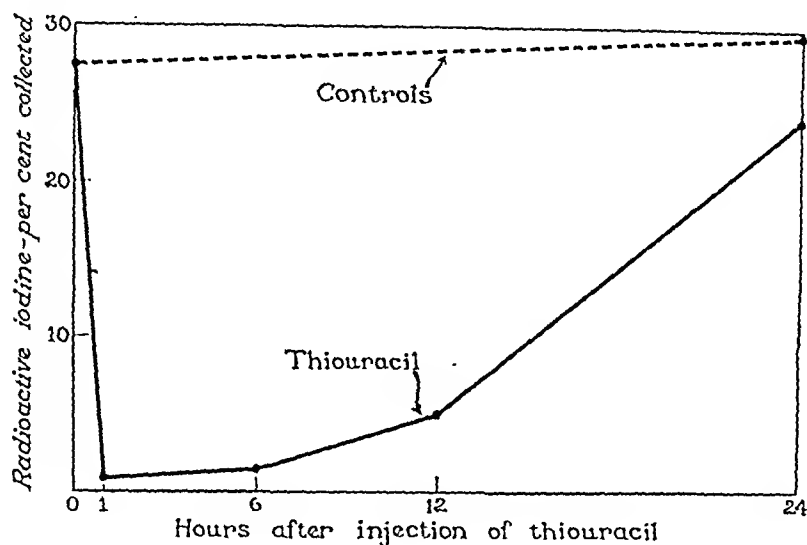


FIG. 8. The effect of thiouracil administered by subcutaneous injection on the collection of radioactive iodine by the thyroids of baby chicks. The chicks received four daily injections of thyrotropin, one unit daily. On the fifth day 10 mg. of thiouracil was administered at zero hour and radioactive iodine was administered at 1, 6, 12 and 24 hour intervals after the thiouracil had been administered. Radioactive iodine was administered to controls, which had received thyrotropic hormone only, at zero hour and 24 hours later. Each point on the figure represents the average per cent of the administered radioactive iodine recovered in the thyroids of each group.

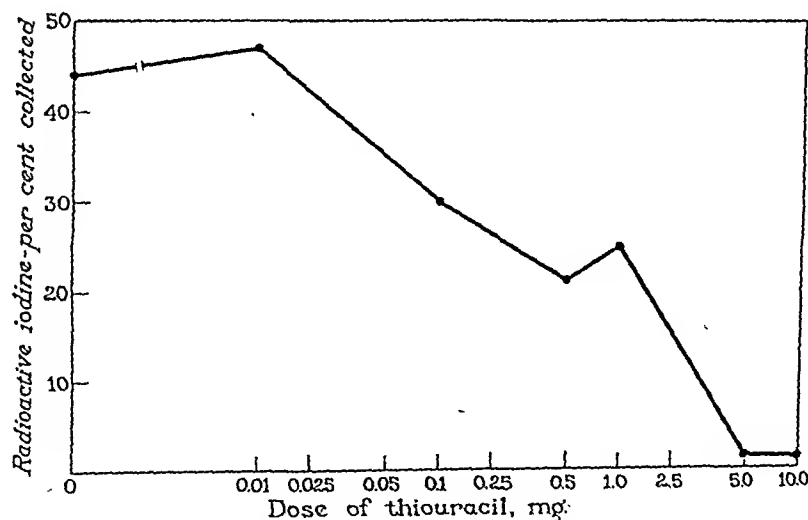


FIG. 9. The effect of varying doses of thiouracil administered subcutaneously on the collection of radioactive iodine administered one hour after the thiouracil had been injected. The chicks were treated with four daily injections of thyrotropic hormone, one unit daily, and the thiouracil was administered on the fifth day. Each point on the figure represents the average per cent of the administered radioactive iodine recovered in the thyroids of each group.

the test agent. Chicks were treated with thyroid stimulating hormone for 4 days and received the test agents in varying amounts on the 5th day. The radioactive iodine was administered one hour later. The per cent block to the collection of iodine was determined by the formula

$$100 = \frac{\% \text{ administered iodine collected by thyroids of test animals}}{\% \text{ administered iodine collected by thyroids of control animals}}$$

The drugs used included thiouracil, 6 n propyl thiouracil, benzyl thiouracil, 5 aminothiazole -2- thiol, 3 (phenyl-aminomethyl) thiazolidine -2- thione, aminothiazole and potassium thiocyanate.

In the rat it was observed that the relative effectiveness of these agents in producing a block to the collection of radioactive iodine descends as follows: benzyl thiouracil, propyl thiouracil, thiouracil, 5 aminothiazole -2- thiol and 3 (phenyl-aminomethyl) thiazolidine -2- thione. In the chick these agents produced a block to the collection of iodine in the following descending order: benzyl thiouracil, propyl thiouracil, thiouracil, 5 aminothiazole -2- thiol, potassium thiocyanate, aminothiazole and 3 (phenyl-aminomethyl) thiazolidine -2- thione.

The same investigators (52) in a parallel chronic study determined the capacity of chick and rat thyroids, made goitrous with the same agents, to collect radioactive iodine. In the goitrous rat the collection of iodine per mg. of tissue in all instances was less than in the controls. The order of capacity to produce a block descended as follows: 3 (phenyl-aminomethyl) thiazolidine -2- thione, thiouracil, 5 aminothiazole -2- thiol, aminothiazole and potassium thiocyanate. In the chick quite different results were obtained. A marked decrease in the collection of iodine per mg. of tissue was observed in animals treated with benzyl thiouracil, propylthiouracil and thiouracil. However, the collection of iodine per milligram of thyroid tissue in chicks made goitrous with aminothiazole, 3 (phenyl-aminomethyl) thiazolidine -2- thione, 5 aminothiazole -2- thiol and potassium thiocyanate was significantly greater than that collected by the controls.

While phenylaminomethyl 2 mercaptothiazoline and 5 aminomercaptothiadiazole in the rat are equal to thiouracil in goitrogenic activity, they are not equal in their capacity to block the collection of radioactive iodine. Therefore, it might be concluded that no necessary correlation exists between the goitrogenic and iodine blocking action of these agents.

The iodine collecting capacity of thyroids made goitrous with various agents appears to be at least partly a function of the time elapsing after the last administration of the agent. This is indicated by the work of Chaikoff and Taurog and of Randall and Rawson. Chaikoff and Taurog (53) have observed that the iodine concentrating capacity of the intact thyroid is depressed in rats treated with potassium thiocyanate provided high con-

centrations of the drug are still present in the circulation. They have also found that 16–24 hours after the last administration of potassium thiocyanate, when potassium thiocyanate can no longer be demonstrated in the plasma, the enlarged gland has a greater than normal capacity for concentrating radioactive iodine. Randall and Rawson (54) have observed a decreased capacity of the thyroids of rats and chicks to concentrate radioactive iodine administered one to six hours following the subcutaneous injection of 10 mg. of potassium thiocyanate. The block was no longer demonstrable after 12 hours.

Radioactive iodine has proved useful in studying the effect of thyroxine on the thyroid. Loeser (55) has postulated that the decreased response of thyroxine treated guinea pigs to injections of thyrotropic hormone is due to a depression of the animal's own pituitary. However, another possibility is that the exogenous thyroid hormone might exert a depressing effect through some direct action of the thyroid cell. With this question in mind, Cortell and Rawson (56) have investigated the effect of thyroxine on the response to thyrotropic hormone in hypophysectomized rats. The response to the thyrotropic hormone was determined by thyroid mean acinar cell height measurements and by the collection of radioactive iodine. Two groups of 19 hypophysectomized rats were studied. One group served as a control; the other group was treated with 20 gamma of thyroxin daily for ten days. Each group of animals was divided into three subgroups, and on the seventh, eighth, ninth and tenth days the animals in each subgroup received 0, 4, and 8 units (26) of thyrotropic hormone respectively. On the 11th day all animals were sacrificed. Their thyroids were examined microscopically under oil immersion. Treatment with thyroxine alone failed to produce any changes in the mean cell height of the hypophysectomized rat thyroids. The mean acinar cell height of animals treated with 4 units of thyrotropic hormone for 4 days was 6.5 micra; of animals treated with the same dose of thyrotropic hormone plus thyroxine, 4.8 micra. The mean acinar cell heights of animals treated with 8 units of thyrotropic hormone was 7.5 micra; of animals treated with this dose of thyrotropic hormone plus thyroxine 6.5 micra.

The capacity to collect radioactive iodine was determined in similarly treated rats. Four groups of hypophysectomized rats were studied. The first group received thyroxine 20 gamma daily for 10 days. The second group was treated with thyroxine 20 gamma daily for 10 days and received 4 units daily of TSH on the seventh, eighth, ninth and tenth days. The third group received TSH only; four units being injected on the seventh, eighth, ninth and tenth days. The fourth group received no treatment. A fifth group served as intact untreated controls. Unoperated untreated control rats were found to collect an average of 6.98 per cent of the adminis-

tered iodine, while hypophysectomized untreated rats fixed only 0.43 per cent, an amount which was not further depressed by the administration of thyroxine. The thyroids of hypophysectomized rats treated with thyrotropic hormone fixed 7.8 per cent of the administered iodine. When the

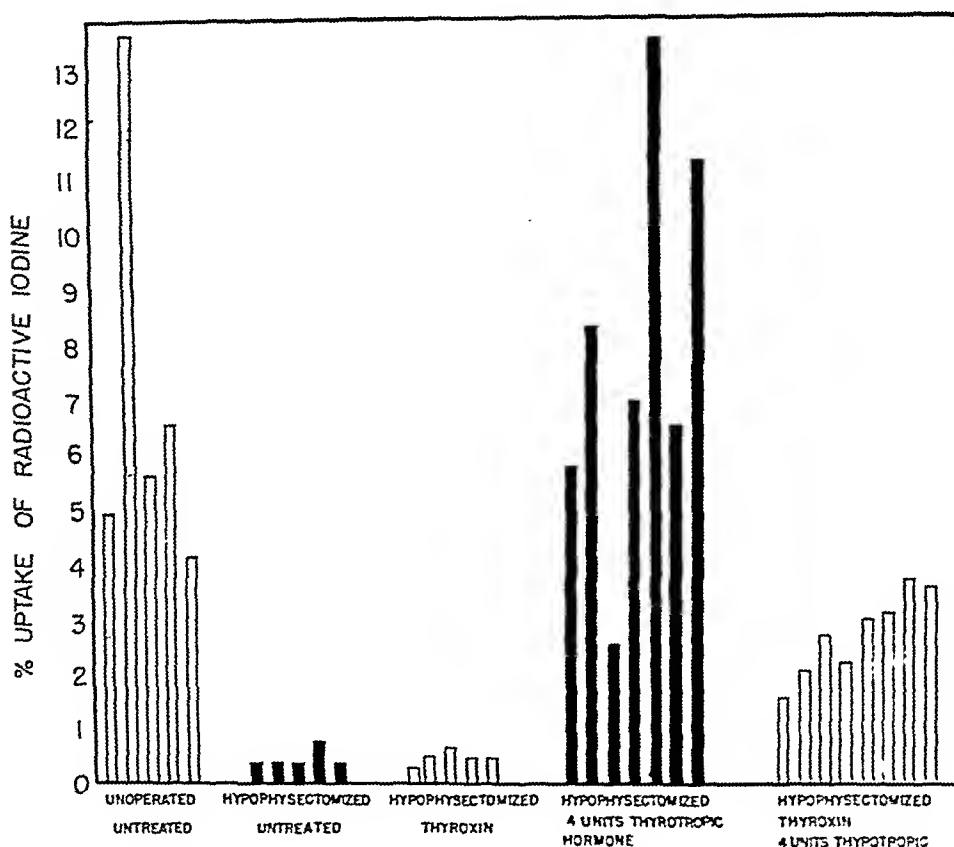


FIG. 10. The effect of thyroxine on the response of the hypophysectomized rats' thyroids to thyrotropic hormone as indicated by the per cent uptake of administered radioactive iodine by the rats' thyroid. Each column represents the per cent uptake of radioactive iodine by a single animal's thyroid gland. Twenty gamma of thyroxine were given daily for 10 days. Thyrotropic hormone was given daily for the last 4 days of the experiment.

thyroxine was administered to hypophysectomized rats along with the thyrotropic hormone the capacity of the thyroid to collect radioactive iodine was depressed to an average of 2.7 per cent (Figure 10). Thus it appears that the administration of thyroid hormone not only depresses the elaboration of the thyrotropic hormone by the pituitary but that it also inhibits the action of the thyrotropic hormone on its end organ.

### The Use of Radio Iodine in the Study of Human Thyroid Disease.

Hamilton and Soley (57, 48, 59) were the first investigators to report the use of radioactive isotopes in tracing the course of iodine given to human beings. They compared the uptake of labelled iodine by the thyroids of normal controls and with that of patients with various thyroid diseases. These measurements were made by placing a Geiger counter tube over the isthmus of the thyroid gland and measuring the gamma rays emitted from the radioactive iodine that had been collected in the thyroid. They also studied the urinary and fecal excretion of the administered radioactive iodine over a period of five days. They observed that iodine given orally was absorbed rapidly from the gastrointestinal tract and that it was demonstrable in the thyroid within twenty minutes of the time of ingestion. A negligible amount was excreted in the feces. They observed that normal individuals, during a five day period, had a urinary excretion of 80 per cent of the administered dose. In the first twenty-four hour period 50 to 60 per cent was excreted. Myxedematous patients excreted, during the five day period of observation, 90 to 95 per cent of the administered iodine. These patients excreted only 45 to 50 per cent of the labelled dose in the first twenty-four hour period. Thyrotoxic patients previously treated with iodine excreted about the same percentage of the administered iodine as did normal individuals. One untreated thyrotoxic patient excreted much less iodine. In another study these same investigators observed that hypothyroid children (60) without goiters collected very little iodine. Two hypothyroid children with large goiters collected relatively larger amounts of iodine in the thyroid. These thyroids when biopsied showed marked hyperplasia.

Hertz, Roberts and Salter (61) have used radioactive iodine in studying the iodine metabolism of patients with Graves' disease. They have administered labelled iodine to thyrotoxic patients and determined the uptake of the iodine by direct in situ measurements with the Geiger counter tube and by determination of the urinary excretion of radioactive iodine. The operatively removed thyroids were fractionated, according to the method of Harington, after alkaline hydrolysis and separated into diiodotyrosine and thyroxine-like fractions. They have observed that the untreated hyperplastic thyroid of Graves' disease collects 80 per cent or more of the administered iodine if the dose be small. The initial collection by previously iodinated patients, and by normal controls, is smaller. They have also observed that with increasing time intervals after the administration of labelled iodine there is an increasing amount of radioactive iodine in the thyroxine-like fraction of the gland.

Hertz and Roberts (62) have compared the urinary excretion of a tracer dose of radioactive iodine in patients with classic Graves' disease and in

patients with the hyperophthalmopathic type of Graves' disease. Patients with ordinary thyrotoxicosis excreted less iodine in the urine than did patients with the special ophthalmic complication.

Rawson et al (63) have observed the fate of radioactive iodine administered to 10 patients prepared for thyroidectomy by the administration of

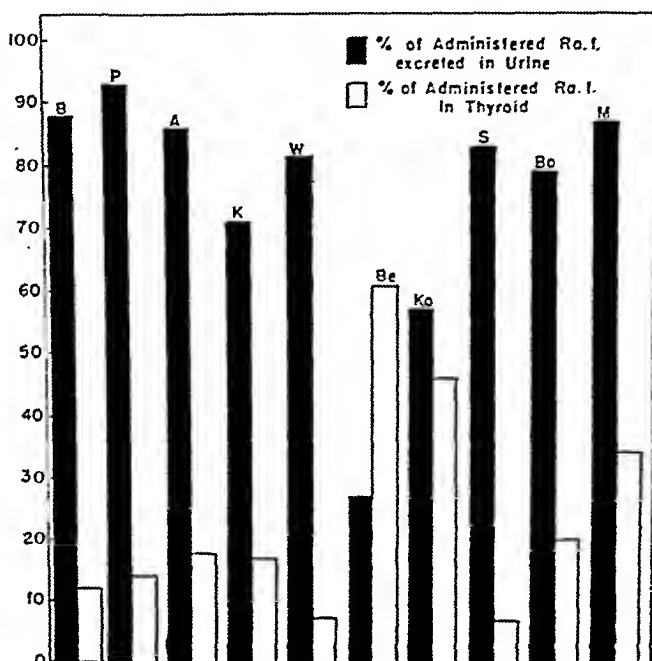


FIG. 11. The excretion in the urine and the collection by the thyroids of radioactive iodine administered to patients with Graves' disease after being prepared for thyroidectomy by the administration of thiouracil. The radioactive iodine was administered 24 to 48 hours before operation. The black columns represent the per cent of the administered radioactive iodine excreted in the urine and the white columns represent the per cent of administered radioactive iodine demonstrated in the operatively removed thyroids.

thiouracil. A tracer dose of radioactive iodine was administered 24 to 48 hours before operation. The radioactivity excreted in the urine and that present in the operatively removed thyroids was determined. In 9 of the 10 cases the greater part of iodine was excreted in the urine and a much smaller amount was demonstrated in the thyroid tissue. These findings are contrasted to observations made on five normal individuals whose urinary excretion averaged 68 per cent of the administered iodine and to those made on four untreated thyrotoxic patients whose urinary excretion averaged 16 per cent of the administered iodine. In Figure 11 is illustrated



the percentage of an administered dose of radio iodine excreted in the urine and the percentage demonstrable in the operatively removed thyroid following treatment with thiouracil.

In another study, Rawson et al. (64, 65) used radioactive iodine tracer techniques in patients with Graves' disease undergoing treatment with thiouracil and thiouracil plus iodine. The purpose of this study was to attempt a clarification of the paradoxical but beneficial effect of iodine on hyperthyroid patients whose thyroids are avid for iodine and are rapidly secreting the absorbed iodine as thyroid hormone. Studies were done before any medication was given, after treatment with thiouracil had caused a fall in the basal metabolic rate to a normal level and after iodine had been added to the regime of treatment with thiouracil. Observations included microhistometric studies of biopsy specimens taken before treatment; after treatment with thiouracil, but before iodine; and of glands removed at operation after treatment with both drugs. The urinary excretion of radioactive iodine was determined before and during thiouracil treatment. Total and precipitable iodines were determined in the operatively removed thyroids. The results showed hyperplasia due to the disease before thiouracil treatment, the mean acinar cell height being 12.0 micra. There was a greater degree of hyperplasia after thiouracil treatment, the mean acinar cell height reaching 13.9 micra. However, involution was observed after iodine had been administered in addition to thiouracil, the mean cell height decreasing to 7.2 micra. Before treatment with thiouracil the excretion of radioactive iodine averaged 25 per cent. During thiouracil treatment the average excretion of radioactive iodine was 80 per cent. The average thyroglobulin iodine of glands from patients treated with thiouracil and iodine was 7.0 mg. per 100 Gm. of fresh thyroid tissue. The average thyroglobulin iodine of a comparable series of patients who had been prepared for operation with thiouracil alone was 6.8 mg. per 100 grams of fresh thyroid tissue; of normal human thyroids 29.8 mg. per cent; of a small series of iodine treated patients 36.6 mg. per cent. All of the iodine administered to 1 patient was labelled with radioactive iodine and thus a balance study was made. Before treatment she excreted only 16.3 per cent of a tracer dose. After she had received thiouracil 0.8 Gm. daily for 15 days she excreted 73.5 per cent of an identical dose of radioactive iodine. Following the second biopsy taken from her thyroid, she received a daily dose of 300 mg. of sodium iodide labelled with 100 microcuries of radioactive iodine. The radioactivity demonstrated in the urine excreted during and after the 10 day period of treatment with thiouracil and labelled iodine practically equalled the radioactivity administered. (See Figure 12). The radioactivity demonstrated in the operatively removed thyroid was reported as too little to measure. Although none of the administered iodine was collected

by the thyroid the administration of iodine did cause an involution of the hyperplastic thyroid. (See Figure 13.) It was concluded that iodine exerts two actions on the thyroid gland, an iodinating action and an involuting action in Graves' disease and that these two actions can be separated one from the other by means of thiouracil.

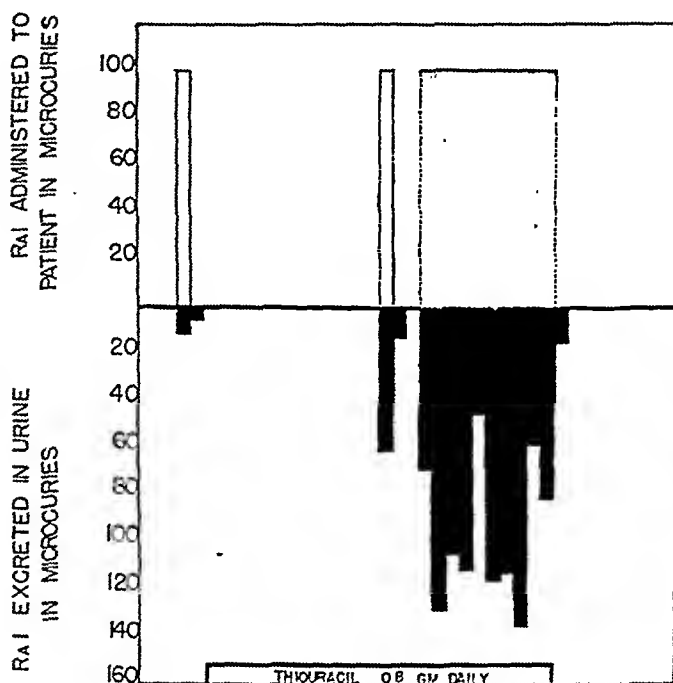


FIG. 12. Radio iodine balance study in patient prepared for thyroidectomy by the administration of thiouracil until the BMR fell to a normal level, then treated with continued thiouracil and added iodine. Intake above the line. Urinary excretion below. With regard to intake, plain columns indicate that tracer iodine was given with carrier of 150 micrograms of sodium iodide. Stippled columns indicate that tracer iodine was given with carrier of 300 mg. daily of sodium iodide. The latter carrier amounted to a therapeutic dose of ordinary iodine.

### The Treatment of Hyperthyroidism with Radioactive Iodine.

In 1942 Hamilton and Lawrence (66) reported that they had produced almost complete destruction of the thyroid in dogs and rabbits with radioactive iodine (eight day isotope) without evidence of damage to other tissues of the body. Smaller doses of radioactive iodine were administered to three patients with hyperthyroidism. Two of the patients were in complete clinical remission four and one half months later and the third had required an additional dose of radioactive iodine.

At the same time Hertz and Roberts (67) described the treatment of ten hyperthyroid patients with radioactive iodine. In a supplementary report (68) these authors described the use of radioactive iodine in the treatment of 29 patients with Graves' disease. Doses of 0.7 to 28 millicuries of the 12 hour isotope of radioactive iodine were given, followed by the usual therapeutic dose of saturated solution of potassium iodide for a period of two to four months. Complete remission of the hyperthyroidism is said to have taken place in 20 of the patients. Owing to the subsequent use of



FIG. 13. Histologic preparations of thyroid biopsy and operative specimens before therapy, after therapy with thiouracil had caused a fall in BMR to euthyroid level and after continued thiouracil plus added iodine.

A. Biopsy before medication. Mean cell height  $15.3 \pm 0.14$  micra.

B. Biopsy after treatment with thiouracil. Mean cell height  $17.3 \pm 0.17$  micra.

C. From operatively removed thyroid after treatment with thiouracil plus iodine. Mean cell height  $10.3 \pm 0.07$  micra. Thyroglobulin iodine 2.3 mg. per 100 gm. of wet tissue.

ordinary iodine, these results cannot command unequivocal acceptance.

Chapman and Evans (69) have also reported on the therapy of Graves' disease with radioactive iodine. They administered radioactive iodine (12 hour isotope) in doses of 14 to 79 millicuries to 22 patients with hyperthyroidism. No additional therapy was employed. Fourteen patients responded well to a single dose of radioactive iodine; three were given two doses and five required three doses. Two patients showed improvement but still had mild hyperthyroidism. Myxedema developed in four patients. Reactions resembling roentgen sickness were observed in six patients who were given large doses of radioactive iodine. Fibrosis of the thyroid was found in biopsies of two patients after radioactive iodine treatment.

### The Collection of Radioactive Iodine by Thyroid Tumors.

In view of the beneficial effect of radiation from radioactive iodine in Graves' disease the question naturally arises, "Can this agent be used in treating tumors of the thyroid?" Though the literature on this subject is meagre, activity in this field is considerable.

Hamilton, Soley and Eichorn (70) have studied the anatomic distribution of radioactive iodine in normal and cancerous thyroid tissue by making radioautographs of thyroids removed from patients two days after the administration of labelled iodine. The stained sections were compared with shadows produced by emanations from the deposited radioactive iodine. They observed that practically none of the iodine is deposited in cancerous tissue. The iodine collected by the normal tissue is deposited in the colloid.

Frantz, Ball, Keston and Palmer (71, 72) have administered tracer doses of radio iodine to three patients having thyroid tumors with bony metastases. The uptake of iodine was determined by means of the Geiger counter. The colloid-containing bony metastases of two of the tumors (one, a Hürthle cell tumor; the other, a well differentiated adenoma malignum) failed to take up radio iodine. The third tumor was a carcinoma of the adenoma malignum type with widespread bony and visceral metastases. Only one of the bony metastases showed an appreciable uptake of radioiodine. At autopsy this metastasis was well differentiated histologically, whereas the other metastatic tissue was undifferentiated.

Marinelli and his associates (73) have determined the uptake of radioactive iodine by 19 carcinomas of the thyroid, employing the radioautographic technique. Ten of the tumors took up a detectable quantity of radioactive iodine. Five of these were examples of the "benign metastasizing struma," five had the structure of follicular adenocarcinoma, and one was a highly malignant-appearing solid alveolar carcinoma. In those cases yielding negative results, there were three examples of Hürthle cell carcinoma and the remainder were either solid alveolar or more anaplastic carcinomas. Inasmuch as the tumors studied represent a selected group of cases, the authors emphasize the fact that the figures quoted are somewhat misleading. If the frequency distribution of the various types of thyroid carcinoma be taken into consideration, roughly 15 per cent may be expected to accumulate radioactive iodine in some degree. In no case of "benign metastasizing goiter" was a negative result obtained. Nearly the same statement can be made from this study in regard to orderly follicular carcinoma, but the avidity of the cells of this type of tumor for iodine does not approach that of metastasizing struma. There was a striking lack of uniformity in the pick-up pattern of various thyroid carcinomas that are functional.

McArthur and Cope (74) administered radioactive iodine preoperatively to 18 patients with discrete nodules of the thyroid, and compared the radioactivity of digests of the tumor tissue with digests of the adjacent uninvolved tissue. Of the 18 tumors, 12 were benign. They included four involutinal nodules, four struma nodosa micro et macrofolliculare, two fetal adenomas, one papillary adenocystoma and one Hürthle cell adenoma. The iodine collected by the less differentiated tumors, such as the fetal adenomas and the Hürthle cell adenoma, was one fiftieth to one hundredth of that collected by the uninvolved portion of the gland. Four of the differentiated tumors collected more iodine than the uninvolved tissue, one twenty times as much. Two other differentiated tumors absorbed all of the iodine, the uninvolved tissue none; the patients had mild hyperthyroidism. The six malignant tumors included papillary adenocarcinoma and alveolar adenocarcinoma. These collected from none to one fourth of the radioactive iodine taken up by the adjacent uninvolved tissue. In three instances there was a detectable collection of iodine by the tumor metastases.

LeBlond and his collaborators (75) compared nodules and surrounding thyroid tissue in regard to iodine content and to the entry of radioactive iodine into the various iodine fractions. Thyroid adenomas were functionally less active than the surrounding uninvolved tissue, as shown by a lower iodine content, a smaller fixation of radio iodine and a slower turnover of the iodine. The difference was greatest in one case of fetal adenoma, but was also demonstrable in varying degrees in four colloid nodules.

Leiter *et al.* (76, 77) have employed radioactive iodine as an indicator of the physiological activity of the metastatic lesions in two patients with adenocarcinoma of the thyroid and hyperthyroidism. Thyroxine iodine was demonstrated in the metastases of both patients. In one patient, the absence of functional thyroid tissue in the neck, shown by the lack of uptake of radioactive iodine, indicated that the metastases were responsible for the hyperthyroidism. Iodinization failed to check the increasing hyperthyroidism of one patient and only slightly decreased the toxicity of the other. Thiouracil induced a clinical remission, decline in the basal metabolic rate and blood protein-bound iodine, and an increase in plasma cholesterol and body weight. There was a prompt recurrence of hyperthyroidism in both patients upon withdrawal of thiouracil. When deep roentgen therapy proved ineffective, several massive doses of radio-iodine were administered to one of these patients. A striking improvement in the clinical picture resulted: the basal metabolic rate dropped to below normal, the patient gained weight and the growth of the metastases appeared to be arrested.

A subsequent report (78) describes in greater detail the radioactive iodine therapy administered to one of these patients, now followed for

three years. When only 30 to 40 per cent of a tracer dose was shown to be excreted in the urine over a 72 to 96 hour period, treatment with radioactive iodine appeared practicable. Radioautographs of a biopsy of one of the tumor metastases after the administration of a large tracer dose of radioactive iodine likewise showed heavy radio iodine concentration in the tumor tissue. A total dosage of 110.8 mc. of 12 hour iodine and 158 mc. of 8 day iodine has been administered to date. Though the patient exhibits marked clinical improvement and arrest in growth of the metastases, tracer studies still show localization of radio iodine in the metastatic lesions. Further therapy is being planned.

From studies thus far reported it seems that therapy with radioactive iodine in thyroid cancer will be possible only in exceptional cases unless means can be found to increase the avidity of thyroid tumors for iodine.

### SUMMARY

Radioactive iodine has proved itself a valuable tool for the study of thyroid physiology. Certain principles of thyroid function which were demonstrated with the classic biochemical techniques have been reenforced and amplified. Progress toward the solution of the steps involved in the synthesis of the natural thyroid hormone has been facilitated. The mode of action of certain goitrogenic thyroid-inhibiting agents has been clarified.

At the clinical level, knowledge of iodine metabolism in Graves' disease has been advanced. Some correlation between the structure and function of benign and malignant tumors of the thyroid has been indicated. In adequate dosage radioactive iodine has proved to be a therapeutic agent of value in certain selected cases of hyperthyroidism and in exceptional instances of thyroid malignancy.

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# THE EFFECT OF TRAUMA AND DISEASE ON THE URINARY 17-KETOSTEROID EXCRETION IN MAN<sup>1</sup>

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IN 1941, in reporting a study of the normal variation in the urinary 17-ketosteroid excretion, Fraser, Forbes, Albright, Sulkowitch and Reifenstein (14) remarked upon a decrease in some of the subjects following various kinds of injury or stress. In a preliminary report a year later, Forbes (13) gave a few more examples of this phenomenon and speculated upon its possible relationship to the "Adaptation Syndrome" described by Selye (26). Since that time we have studied the problem further, and have accumulated a substantial body of data which it is the purpose of this article to present.

The method of total neutral 17-ketosteroid assay employed in these studies has already been described (Fraser, *et al.* (14)). The sources of the steroids measured are the testis and the adrenal cortex in males, and the adrenal cortex alone in females. Hence, the test provides a chemical measurement which is an index, especially in females, of at least one aspect of adrenal function.

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<sup>1</sup> Presented in part at the twenty-fifth annual meeting of the Association for the Study of Internal Secretions, Atlantic City, N. J., May 3, 1941. Preliminary reports of part of this material have been given elsewhere: (1) Albright, F., E. C. Reifenstein, Jr., and A. P. Forbes: Conference on the Metabolic Aspects of Convalescence (Including Bone and Wound Healing), 4th Meeting, New York, June 11-12, 1943; Transactions distributed by the Josiah Macy, Jr., Foundation; pp. 152-165; (2) Forbes, A. P.: The 17-Ketosteroid Excretion in Stress. Preliminary Report. Booklet distributed by the Josiah Macy, Jr., Foundation, New York, June, 1942; and (3) Reifenstein, E. C., Jr.: Conference on Ketosteroids, Hotel Seaside, Atlantic City, N. J., June 7, 1942. Booklet distributed by the Josiah Macy, Jr., Foundation, New York, pp. 24-25; also in *J. Clin. Endocrinol.* 3: 301-303, May, 1943.

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During a clinical survey involving some 4,000 determinations of the urinary 17-ketosteroid excretion of normal and abnormal people, we observed that the output of chronically ill or severely undernourished patients is remarkably low. We found also that members of the laboratory staff, whose basal excretion was well known and very constant, sometimes put out as little as 30 per cent of their normal amount following an acute infection. Figure 1 shows the behavior of the urinary 17-ketosteroid level during an episode of subacute appendicitis which developed in one worker whose urine we happened to be assaying daily. The lowered steroid output appeared before the symptoms, and constituted the first evidence of ill-

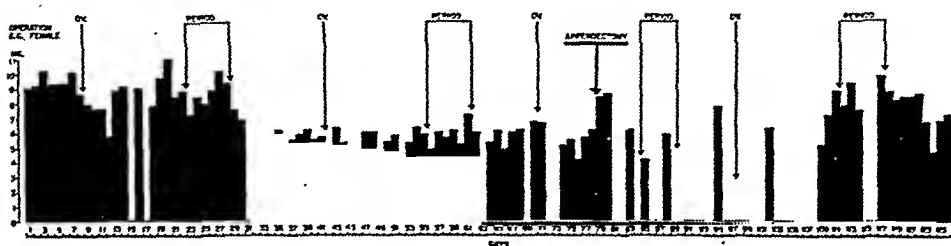


FIG. 1. The urinary 17-ketosteroid excretion of a normal woman during an episode of subacute appendicitis.

ness. Operation caused an abrupt peak in the output followed by a drop to an even lower level than before and a gradual return to the baseline during convalescence. Figure 2A, 2B, and 2C show the findings in three normal individuals undergoing operation, childbirth, and an acute infection, respectively. The line marked "Alarm" represents the day of the injury or of the onset of the disease. These last three charts do not show the initial peak seen in Figure 1 possibly because assays were not made every 24 hours, but all of them are similar in that the 17-ketosteroid excretion is eventually markedly depressed.

These early observations led us to believe that the 17-ketosteroid excretion might be regularly affected by any sort of damage to the body and might be employed to obtain valuable information about the function of the adrenal cortex following damage. With this in mind, we reviewed the data already in our files and began to add to them at every opportunity. We now have studies of the urinary 17-ketosteroid excretion of more than one hundred patients who have been followed through some sort of medical or surgical injury.

### I. The 17-Ketosteroid Excretion of Normal Subjects.

We have made assays on 73 normal young men and 65 normal women with regular menstrual cycles. The excretion of normal men fell between

the extremes of 6.7 and 27.2 mg. of steroid per 24 hours; and those of the women between 3.8 and 16.9 mg. per 24 hours. The average for men was 12.5 and for women 8.2 mg. per 24 hours. These values correspond fairly closely with those for normal individuals reported by other investigators (Chou and Wang (9); Callow, Callow, Emmens, and Stroud (8); Fried-

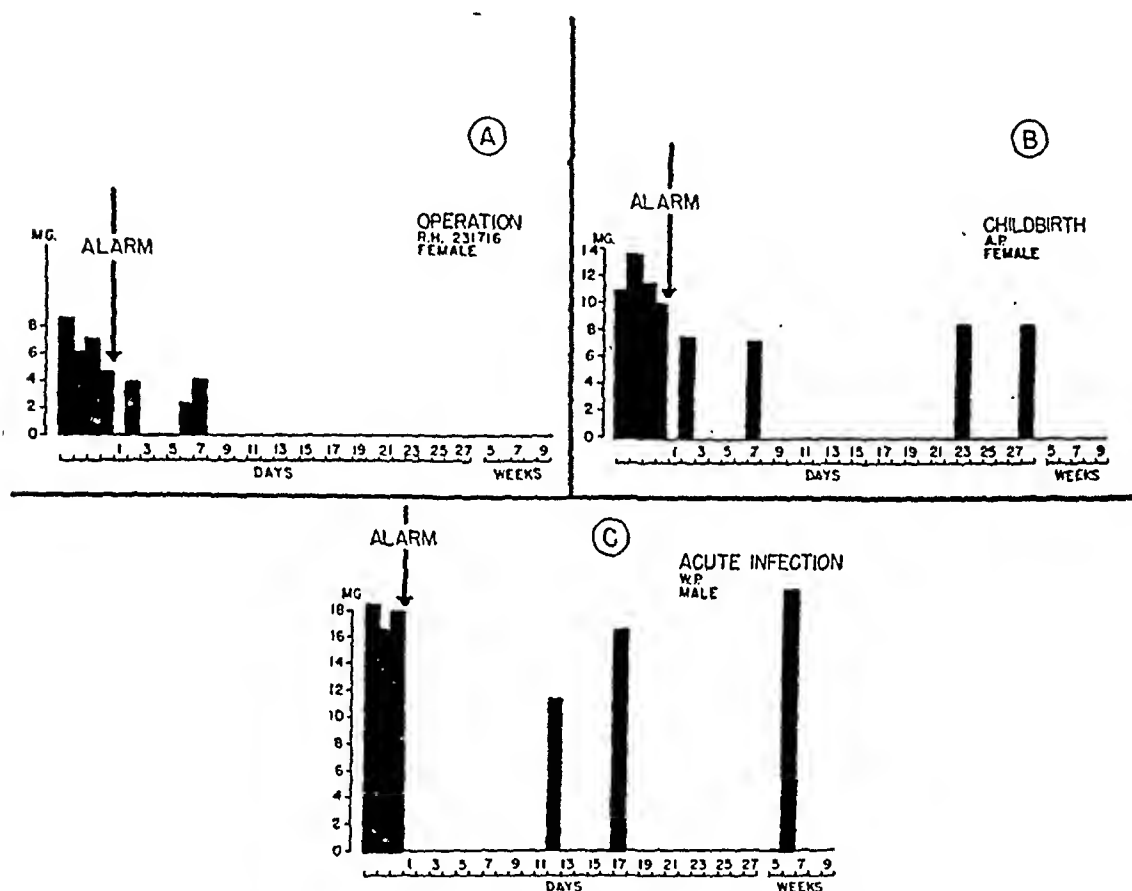


FIG. 2. The urinary 17-ketosteroids of three normal individuals undergoing operation (A), childbirth (B), and acute infection (C).

good and Whidden (17); Baumann and Metzger (5); Talbot, Butler, Berman, Rodriguez, and MacLachlan (30); Werner (32); and Engstrom and Mason (12)). We do not have extensive data on the relationship of the 17-ketosteroid excretion to age; what little we do have show that the level in adults decreases somewhat with increasing age but not markedly until well after the climacteric (Fraser, *et al.* (14)). We could not establish any significant relationship to height, weight, complexion or creatinine excretion.

The amount of scatter between apparently similar individuals is very great and rather baffling. For instance, within a group of 30 student nurses who had all passed medical examinations and in whom the age, diet, and

activity were all quite uniform 47 assays varied from 3.8 to 16.9 mg. per 24 hours (average 7.5 mg.) with a standard deviation from the mean of 3.4 mg. Although the extremes may represent inaccurate collections or minor illnesses, the wide variation indicates that a single 17-ketosteroid determination must be very high or very low to have any diagnostic value.

The results of repeated assays on the same individual are much more consistent. In 17 of the nurses just mentioned, on whom the assay was repeated, the standard deviation between the first and the second determinations was 3.0 mg. in contrast to the 3.7 mg. above. Similarly, in seven normal laboratory workers, who brought in daily collections for one month, the standard deviation from the mean of each individual series was less than 2.5 mg. Five of these subjects made separate collections during their sleeping and waking hours. Figure 3A and 3B show two such experiments, one in a female and one in a male. These charts indicate that the 24-hour excretion in any one case remains much the same from day to day. They bring out another fact, which Pincus (22) and Bachman, Leekley, and Winter (4) have also observed, namely, that the excretion is lower per hour during sleep than during waking activity. The excretion is also somewhat more constant during sleep. The standard deviation from the mean for the day-collections in the two people illustrated in Figure 3 (see Table 1) is 1.9 mg. for the female (whose average excretion was 8.6 mg. per 24 hours for the day), and 2.3 mg. for the male (whose average excretion was 13.2 mg. per 24 hours for the day). During sleep, the female averaged 7.5 mg. per 24 hours with a standard deviation of 1.2 mg.; and the male averaged 10.3 mg. per 24 hours with a standard deviation of 2.3 mg. This difference obtains in spite of the fact that the sleeping hours were about half as many as the waking hours, so that the results are multiplied by approximately twice as much in calculating the 24-hour level. If two sleeping collections are pooled to make one specimen of approximately 16 hours of collection, the result is very constant. The standard deviations (see Figures 3A and 3B) then become 0.8 and 1.9 mg., respectively. The same fact appears on analyzing the other charts. The values in females suggest a slight drop in excretion at the time of ovulation, but we have not made any special allowance for this. Table 1 summarizes the results on all seven subjects, and leads to the conclusion that the sleeping excretion is 12 to 22 per cent lower than the waking, and almost twice as constant. Table 2, previously reported elsewhere by Reifenstein (23) contains confirmatory data analyzed in terms of groups rather than individuals.

Wooster (34) made a statistical comparison of the sleeping excretion calculated on a 24-hour basis with the total 24-hour excretion and found that the sleeping excretion is irregularly lower and more variable than the 24-hour excretion. Werner (33) found that the day to day fluctuations are

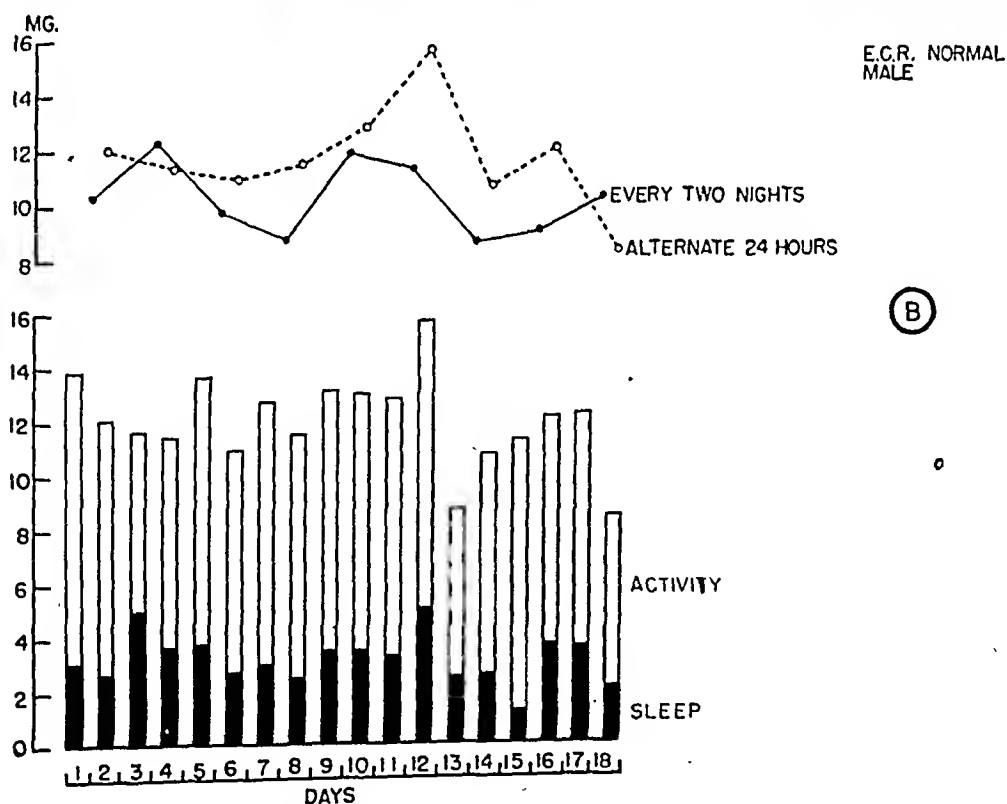
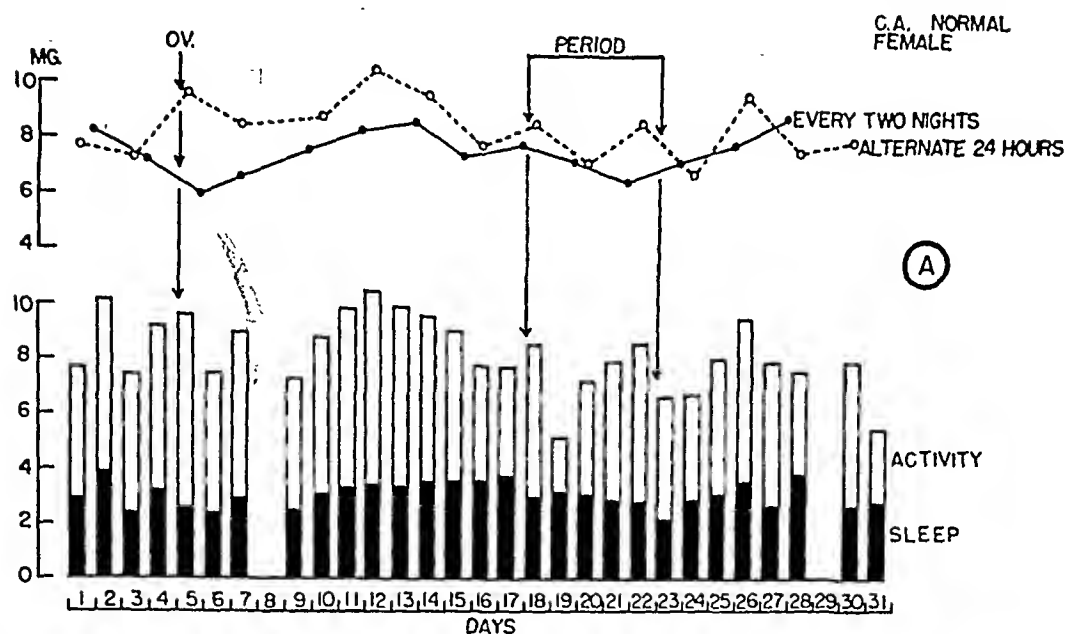


FIG. 3. The urinary 17-ketosteroid excretion per hour during the day and during sleep by a normal woman (A) and by a normal man (B).

TABLE 1. THE WAKING AND THE SLEEPING URINARY EXCRETION OF 17-KETOSTEROIDS IN SEVEN NORMAL SUBJECTS STUDIED DAILY FOR ONE MONTH

Subject	Sex	Average Day Level mg./24 hr.	Average Night Level mg./24 hr.	Difference of Day from Night %	Standard Deviation		
					Day	Night	Two Nights
C.A.	f	8.6	7.5	12.8	1.9	1.2	0.8
E.C.R.	m	13.2	10.3	22.0	2.3	2.3	1.9
E.T.R.	f	8.9	7.7	13.5	1.0	1.3	1.3
E.C.D.	f	8.7	6.8	21.8	1.7	1.1	0.9

Subject	Sex	Average of Daily 16-Hour Collections mg./24 hr.	Standard Deviation
E.C.D.	f	8.1	1.3
R.M.S.	f	7.4	1.3
A.P.F.	f	10.6	1.1

TABLE 2. VARIATION IN THE AVERAGES OF THE WAKING AND SLEEPING URINARY EXCRETION OF 17-KETOSTEROIDS OF A SERIES OF REPEATEDLY ASSAYED INDIVIDUALS

Type of Collection	Subjects			Assays		
	Number	Sex	Age Range yr.	Number of Determinations	Range of Individual Averages mg./24 hr.	Average of Individual Averages mg./24 hr.
16 hours from 4 P.M. to 8 A.M.	10	f	23-37	149	5.7 to 10.7	8.2
	10	m	24-42	46	11.9 to 15.4	13.6
±8 hours while asleep	6	f	24-37	121	5.1 to 10.1	7.5
	5	m	29-42	36	8.2 to 15.7	11.9
±8 hours while awake	3	f	24-31	11	6.5 to 11.5	9.0
	4	m	32-42	23	10.2 to 20.2	15.2
±16 hours while awake	3	f	24-37	62	5.2 to 12.2	8.7
	1	m	32	17	11.4 to 14.4	12.9
Total of all types	12	f	23-37	343	5.6 to 11.1	8.4
	11	m	24-42	124	11.4 to 16.4	13.9



still observed even when the extracts are purified with Girard's reagent T so that only the ketonic fraction is measured.

In conclusion there appears to be a basal ketosteroid excretion which is more or less a constant characteristic of a given individual as is the resting blood pressure. However, the deviations from this basal level brought about by daily activities are not very great. Therefore, although in most of our recent cases two "overnight specimens" have been used instead of one 24-hour collection, any major change in 17-ketosteroid level appears in the 24-hour collection as well, and we have used the latter where it was more convenient to do so.

## II. The 17-Ketosteroid Excretion in Patients with Chronic Debility.

The order of magnitude of the changes in ketosteroid excretion produced in an individual by disease is much greater than the usual daily variation. Moreover, chronically ill or severely undernourished people usually have an excretion that falls well below the normal level in spite of the wide range which must be accepted as normal. Table 3 shows the average daily urinary excretion of 196 medical patients taken without selection from the files and grouped by diagnoses. Although we did not ourselves examine each of these patients and supervise the collections for accuracy, as we did in the individual case studies which follow (*vide infra*), we believe that the deviations from normal are significant and are not explained by faulty collections. The average excretion of all the males in this group is 6.2 mg. per 24 hours as contrasted with the average normal value of 12.5 mg., and of the females 4.4 mg. as contrasted with 8.2 mg. Even when the level of a given patient is not below the minimum found in normals, it may prove to be abnormally low for that individual when further determinations are made after recovery. Table 3 includes some debilitated patients who later improved and were tested again when they were well.

These observations and those previously recorded by Fraser, *et al.* (14) are in accord with those of other investigators who have found the 17-ketosteroid excretion of chronically ill patients to be lower than normal (Chou and Wu (10) and later Chou and Wang (9) in hospital patients; Callow, Callow, Emmens, and Stroud (8) in chronically ill hospital patients; Moore, Miller and McLellan (21) in patients with benign hypertrophy of the prostate; Albright and Stewart (3) in a patient with steatorrhea and deficiency in all of the fat soluble vitamins; and Rhoads, Dobriner, Gordon, Fieser, and Lieberman (24) in patients with non-endocrine carcinoma).

In some metabolic and endocrine disorders where low values have been reported, the decrease is probably not due to the disorder per se but to the

accompanying secondary malnutrition. This may be the explanation of the low values reported by Fraser, *et al.* (14) in anorexia nervosa (see also, Fraser and Smith (15)), diabetes mellitus and hyperthyroidism; by Hamblen, Cuyler, and Baptist (18) in diabetes mellitus; by Miller and

TABLE 3. THE URINARY 17-KETOSTEROID EXCRETION OF 196 PATIENTS WITH CHRONIC DISEASE

Diagnosis	Number of Patients	Range in Age yr.	Average Age	Range in 17-Ketosteroid mg./24 hr.	Average Keto-steroid mg./24 hr.
A. Males					
Malignancy (incl. prostate)	24	55-85	70	3.7-22.0	6.7
Sprue; Diarrhea	3	16-31	24	1.8- 2.9	2.3
Malnutrition; Anorexia	6	26-70	42	2.1- 7.6	4.5
Thyrototoxicosis	2	35-58	47	5.1- 5.3	5.2
Diabetes	6	15-71	45	1.8- 6.6	4.6
Hypertension	3	45-48	46	2.8-13.5	8.2
Rheumatic Disease	7	20-66	46	2.7-11.5	7.5
Other diseases	31	16-71	39	1.8-15.9	6.6
Total or Average	82		44		6.2
B. Females					
Malignancy	5	15-70	48	1.5- 4.7	2.7
Sprue; Diarrhea	9	20-50	32	0.5- 8.3	3.3
Malnutrition; Anorexia	27	15-44	29	2.0-11.5	4.9
Thyrototoxicosis	17	18-60	44	1.3-17.3	5.1
Diabetes	9	28-68	55	2.4- 7.5	5.1
Hypertension	8	17-50	41	1.3- 5.3	2.7
Rheumatic Disease	12	16-48	31	1.2- 7.8	4.5
Other diseases	27	16-71	36	1.2- 9.5	4.2
Total or Average	114		37		4.4

Mason (20) in diabetes mellitus; and by Engstrom and Mason (12) in hyperthyroidism. Talbot and Butler (29) have summarized the situation with the statement that, "In chronic illness from any cause there is a tendency for the 17-ketosteroid output to be moderately lowered."

### III. The 17-Ketosteroid Excretion of Previously Healthy Patients After Acute Trauma.

Whenever possible our studies have been carried out by making daily observations on a few individuals rather than scattered observations on a large group of patients. Figures 4 through 13 show the results of such in-

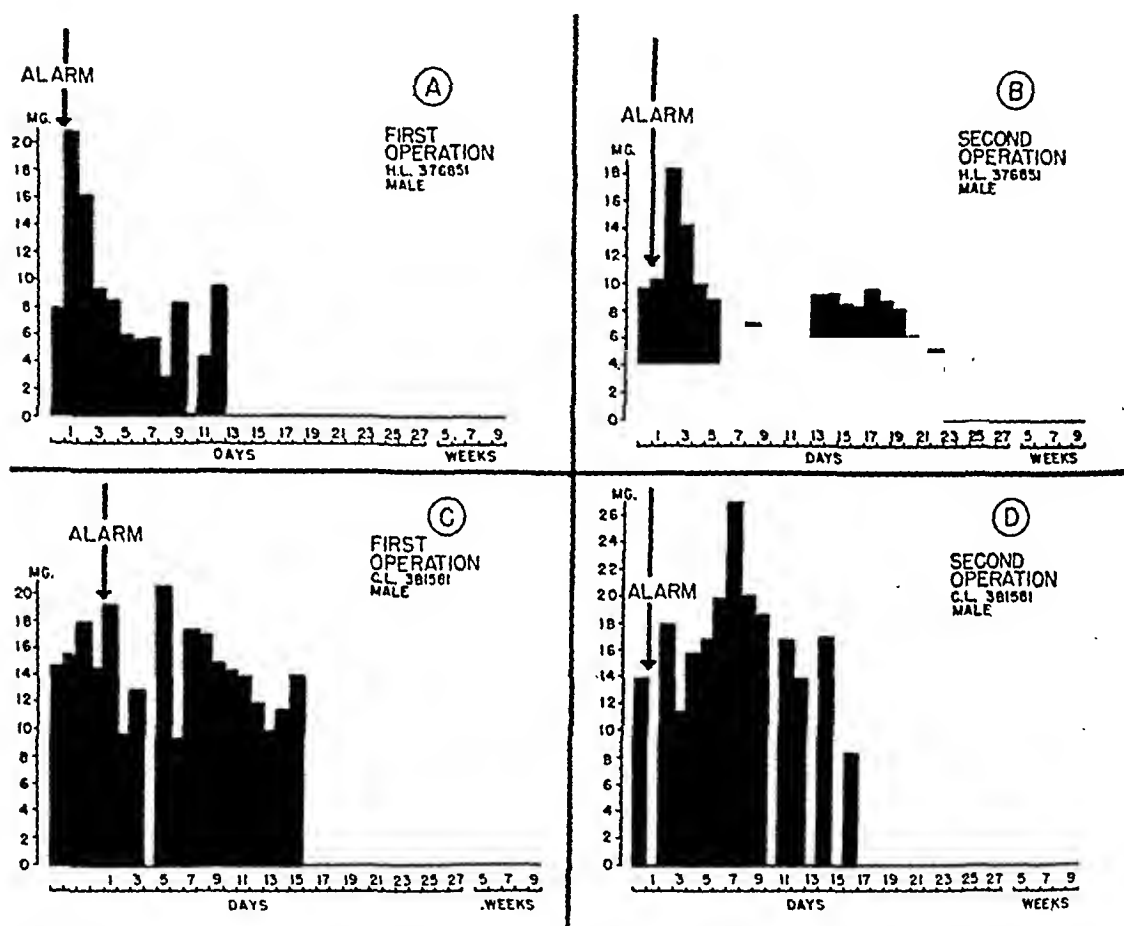


FIG. 4. The urinary 17-ketosteroid excretion of previously healthy men after operation.

Chart A illustrates the changes after the first stage, and Chart B the changes after the second stage of a bilateral lumbodorsal splanchnicotomy for essential hypertension in one individual. Charts C and D show similar data for another patient.

dividual case studies. These charts show that many kinds of trauma and stress affect the ketosteroid excretion according to the same, regular pattern.

Figures 4 through 6 illustrate the effect of surgical operations on previously healthy males. Elective operations were chosen because these allow time for establishing a preoperative baseline. The first three patients (Figures 4A and 4B, 4C and 4D, and 5A and 5B) had bilateral lumbodorsal

splanchnicotomy performed in two stages, the interval between the two operations varying from 12 to 14 days. Thus each of these patients furnishes us with two experiments. These men were 31, 40, and 26 years old, respectively, and except for essential hypertension were in good health. The fourth patient (Figure 5C) had a ruptured intervertebral disc removed.

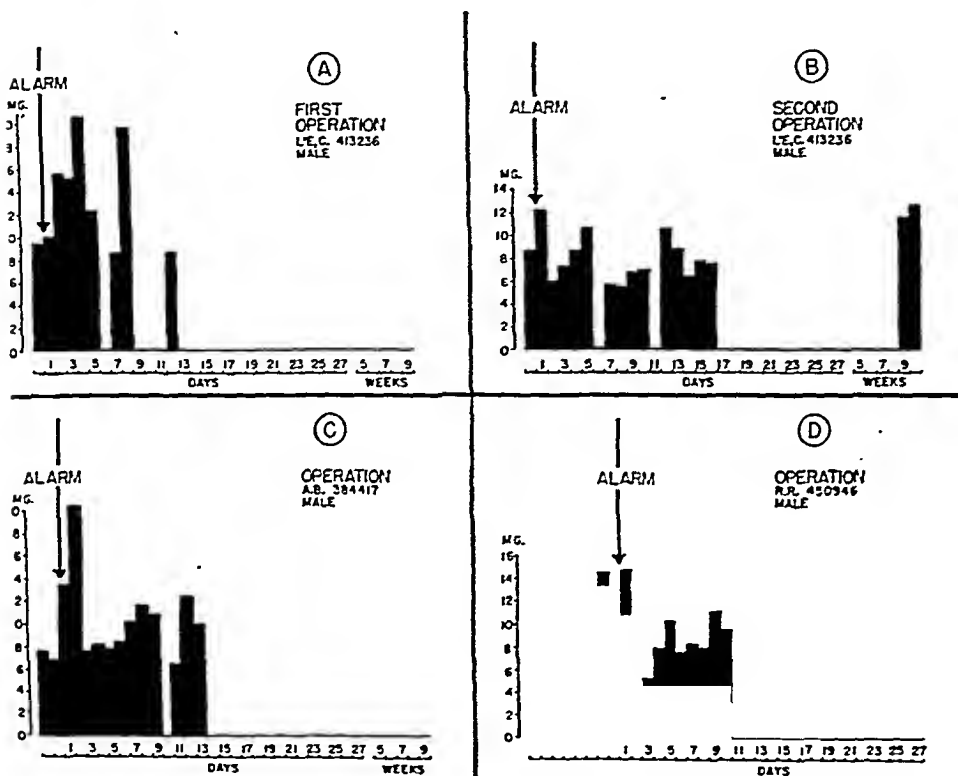


FIG. 5. The urinary 17-ketosteroid excretion of previously healthy men after operation.

Charts A and B represent the changes in the two stages of bilateral lumbodorsal splanchnicotomy of a patient. Chart C illustrates the changes after removal of a ruptured intervertebral disc; Chart D, the changes after a spinal fusion for spondylolisthesis.

He was a 41-year-old football coach, but had been unable to work for about a month because of back pain. The fifth patient (Fig. 5D), a 24-year-old man in good general health and able to chop wood, entered for a spinal fusion for spondylolisthesis. The sixth patient (Fig. 6), a 35-year-old man who was active as a gardener, also entered for a spinal fusion for spondylolisthesis.

The behavior of the 17-ketosteroid excretions was more or less alike in

all of these cases. Within 24 hours to 48 hours after the traumatizing event (operation) there was an increase in the 17-ketosteroid excretion to a point well above the normal limit. This peak usually occurred in the first specimen voided after operation (about 16 hours postoperative) but sometimes did not appear until later. After this the excretion fell rapidly and the low point usually occurred on the fourth or fifth day. Return of the excretion

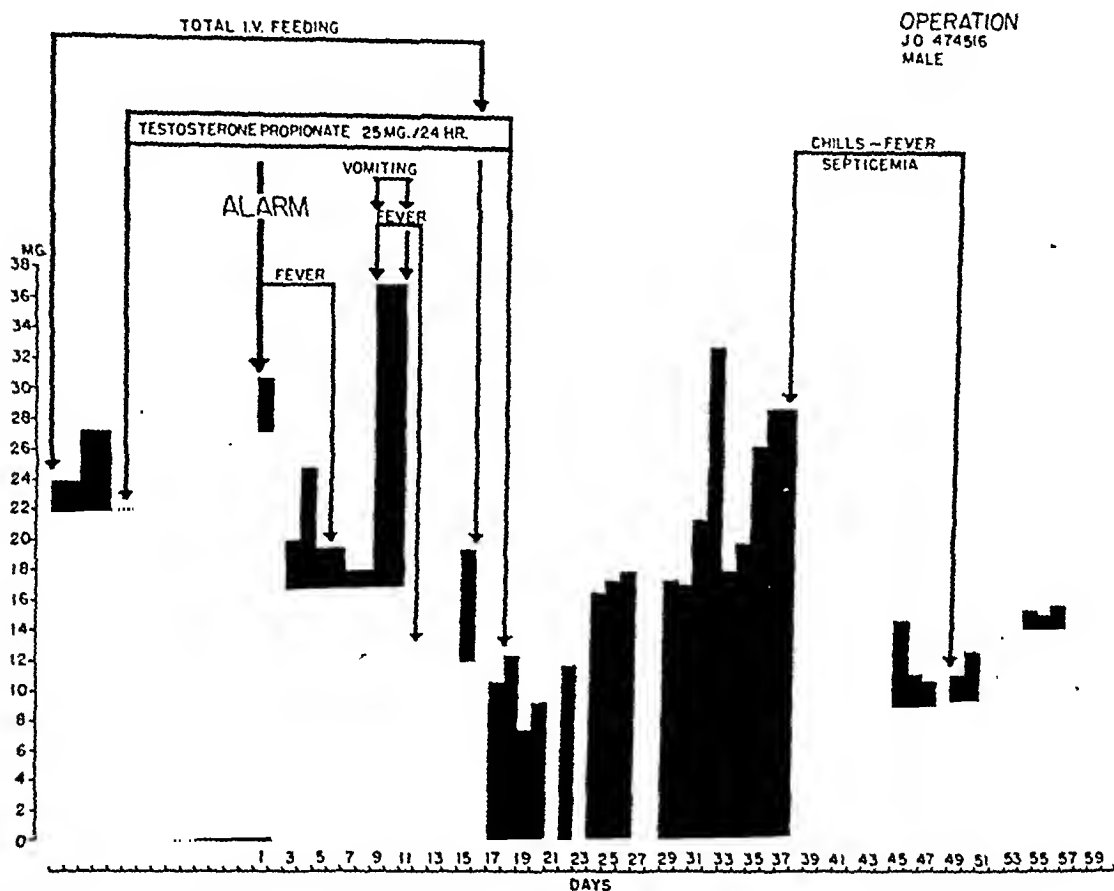


FIG. 6. The urinary 17-ketosteroid excretion after operation of a previously healthy man fed a constant intake and given testosterone propionate.

to a normal level occupied a more variable time, in these cases averaging ten days. Each individual behaved essentially the same way after the second operation as after the first; for instance the patient in figure 4C and 4D whose peak did not occur until the fifth day showed this same very unusual feature the second time. Similarly the patient in figure 4A and 4B showed a typical peak within 48 hours after both operations.

The studies on the fifth and sixth patients constitute strong evidence that the fall in 17-ketosteroids after an operation is not due to decreased food intake. Both were studied on the metabolic ward and both were fed a constant diet throughout entirely by vein. The fifth patient was kept in

bed for 8 days and the sixth for 14 days prior to operation. Their control values are, therefore, more adequate than those of the other four patients studied before and after major surgery. Both of these cases showed the expected pattern of 17-ketosteroid excretion after operation. Since testosterone propionate is partly excreted as a 17-ketosteroid, it should be noted that the sixth patient received 25 mg. of testosterone propionate intra-

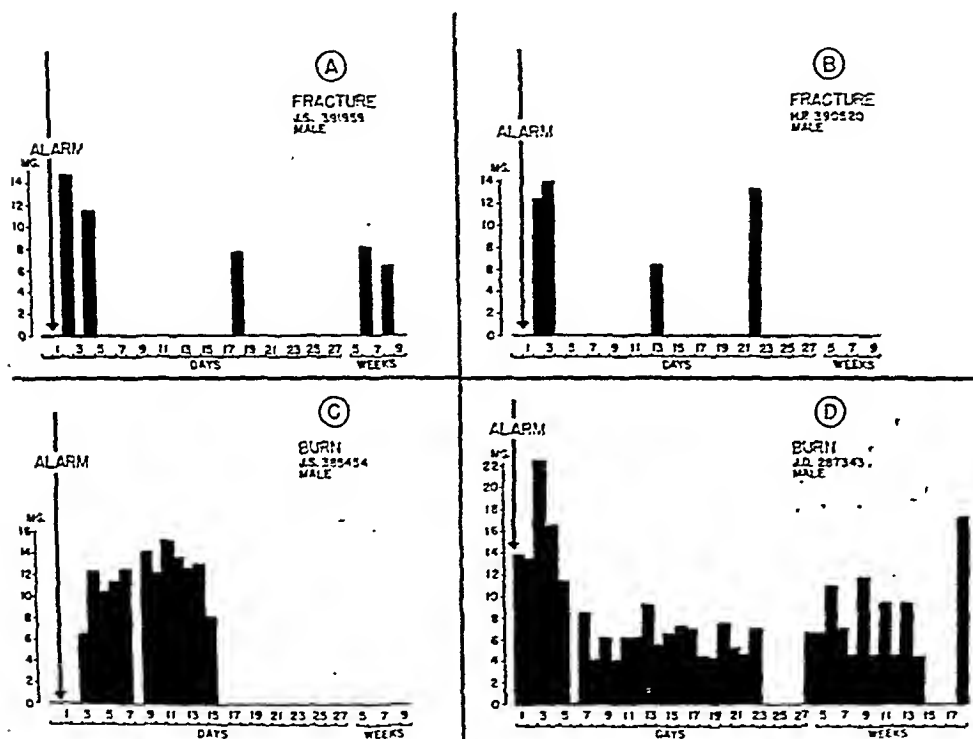


FIG. 7. The urinary 17-ketosteroid excretion of previously healthy men after fracture (A and B), and after burns (C and D).

muscularly each day for 9 days before and for 17 days after operation. Figure 6, in agreement with studies to follow, shows that other types of damaging events (vomiting and fever on days 9 to 11 from too rapid intravenous infusion, and chills and fever from septicemia on days 38 to 48) induce the same alterations in 17-ketosteroid excretion. The fifth and sixth patients have been referred to briefly elsewhere (Albright, Reifenshtein and Forbes (2)).

Figures 7A and 7B show studies on normal males who sustained fractures. In this series there obviously could be no pre-injury control values.

Nevertheless, the curves appear to follow the same general pattern. Assays made before the third day fell in the high normal range and those made after the fifth day fell below normal or at least lower than after recovery. Return from these low levels appeared to be somewhat slower than after the sympathectomies.

The next two cases (Figures 7C and 7D) were brought to the hospital

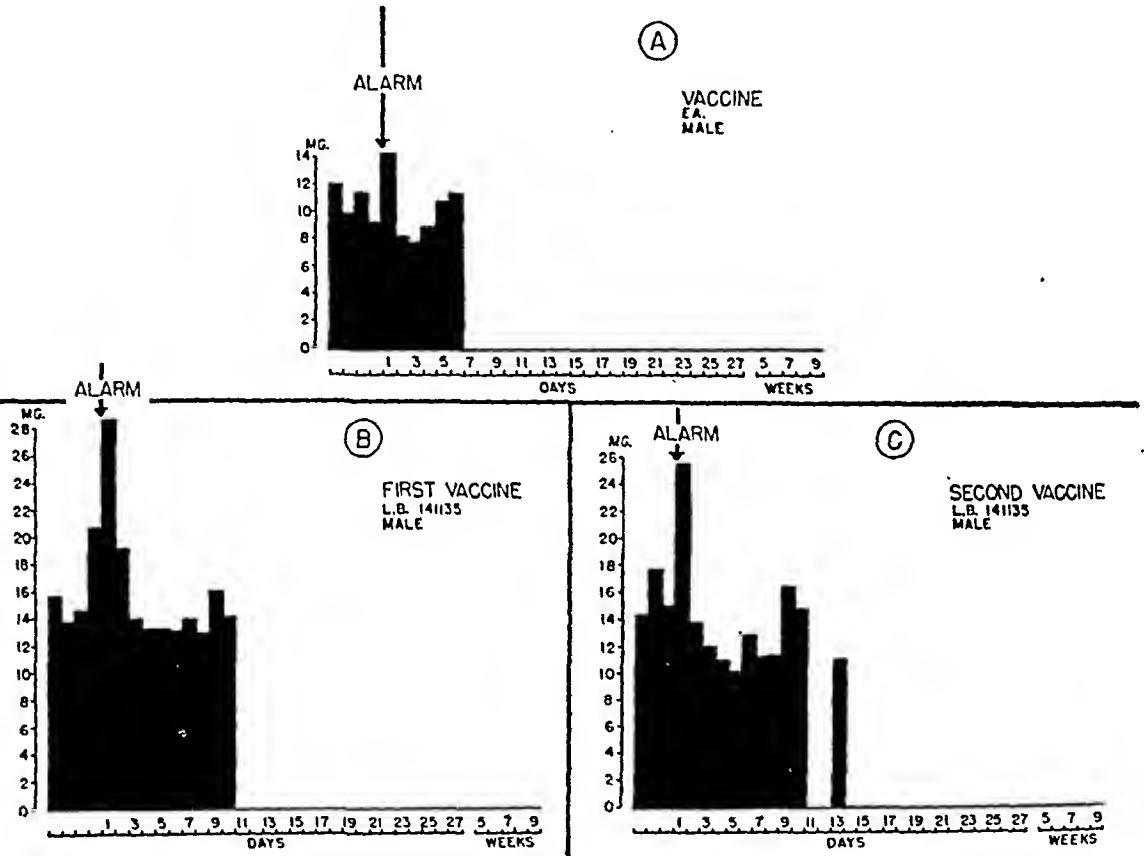


FIG. 8. The urinary 17-ketosteroid excretion of previously healthy men after fever induced by typhoid vaccine.

Charts B and C illustrate changes in two experiments in the same subject performed three months apart.

with burns. The first man was discharged in fifteen days in good condition. His 17-ketosteroid excretion rose from a level of 6 mg. per 24 hours on the third day after the burn to a level of 12 mg. per 24 hours within the first week. The other patient, however, was more severely burned. After an initial peak of 22 mg. per 24 hours on the third day, his excretion fell to between 4 and 7 mg. per 24 hours where it fluctuated for a month, during which time he was very ill. He was discharged after fifteen weeks, but his level was only 4 mg. per 24 hours on the day of discharge. One month later

a follow-up specimen yielded 16 mg. per 24 hours which is entirely normal for a male of this age. These observations agree with those of Cope, Nathanson, Rourke and Wilson (11) who studied these and other patients with burns at the time of the Cocoanut Grove Fire, and also with those of Stevenson, Schenker, and Browne (28) who studied a number of patients following various types of damage (burn, fracture, operation, infection and

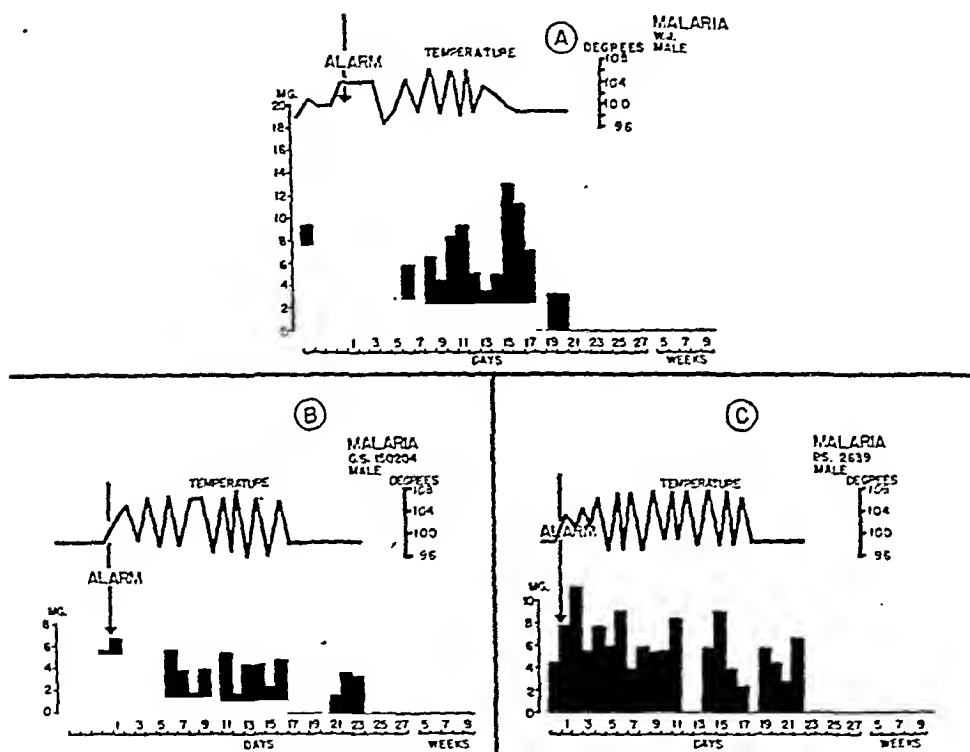


FIG. 9. The urinary 17-ketosteroid excretion of three men after fever due to malarial chills.

hemorrhage) at the Royal Victoria Hospital. The impression is gained from a study of patients with burns that the time required for the 17-ketosteroid excretion to return to normal is definitely related to the nature and severity of the injury and the amount of repair required.

Studies on various medical conditions confirm this impression. Figure 8A shows the effect of a single small injection of typhoid vaccine which produced fever for one day. The pattern of 17-ketosteroid excretion is much like that which follows a surgical injury, but a normal level is regained on the fifth day. Figures 8B and 8C show the results of giving much larger



doses of vaccine intravenously every day for three and for four days, respectively. The 17-ketosteroid excretion came back to normal on the ninth and tenth days. The last two experiments were performed on the same subject with an interval of three months between them. The subject was a healthy thirty-two year old man and the studies were carried out on the metabolic ward where his diet, activity and fluid intake were kept strictly

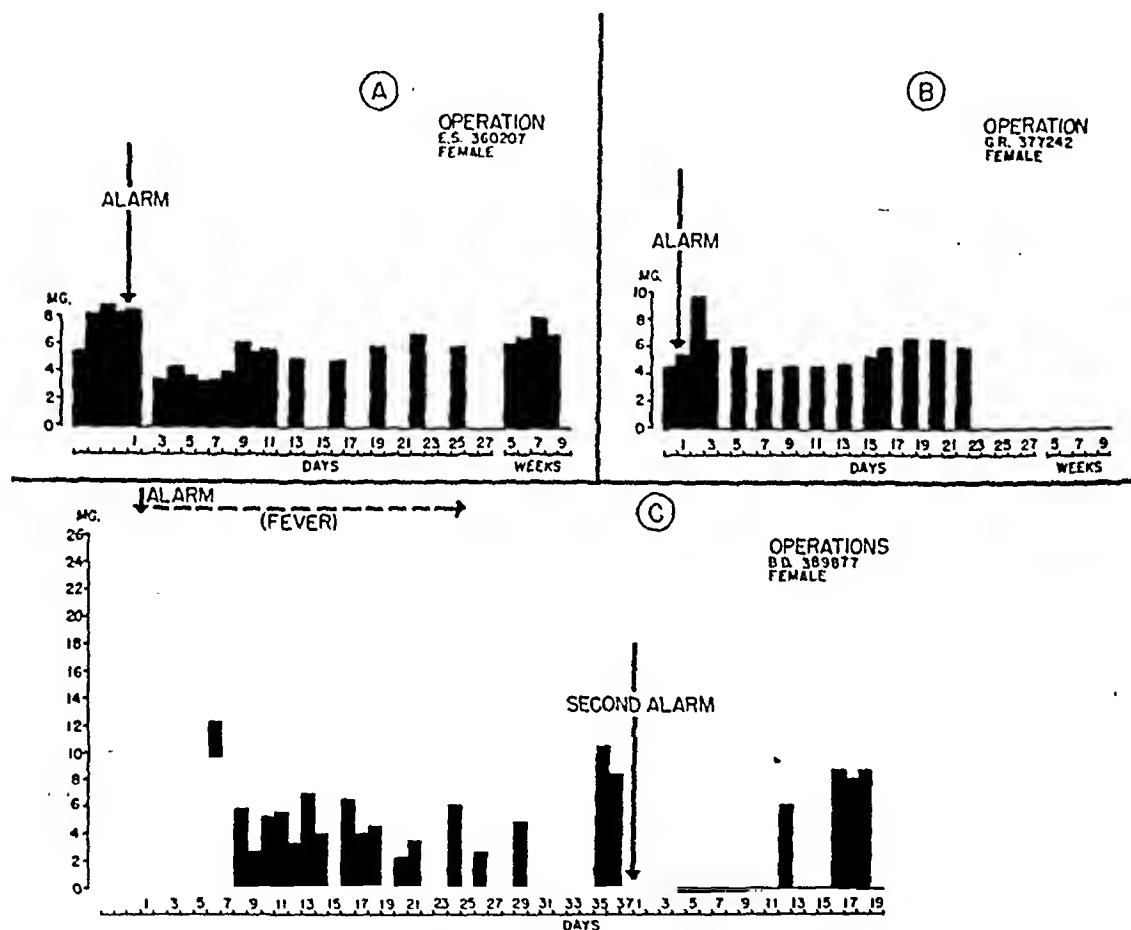


FIG. 10. The urinary 17-ketosteroid excretion after operation of two women who were normal (A and B) and of one woman who had obesity, hirsutism, and irregular menses (C).

constant. In a male laboratory worker who had influenza, the return to a normal level took over two weeks (see Figure 2C).

Since in spontaneous infections we seldom had an opportunity to establish the level of excretion before the infection began, we made some studies on patients who received malarial blood for cerebrospinal syphilis. Figure 9A shows the 17-ketosteroid excretion of a thirty-six year old man with tabes dorsalis who was in good general physical condition before treatment. The first malarial chill affected the 17-ketosteroid excretion in much the

same way as a surgical operation would have. Subsequent chills produced much less reaction and sometimes none. The pattern resembles that found by Howard and Bigham (19) to occur in the nitrogen loss of similar patients. This patient was severely prostrated by the malaria, and quinine was given on the thirteenth day of the disease because of debility. In two other patients (Figures 9B and 9C) there was a peak of 17-ketosteroid ex-

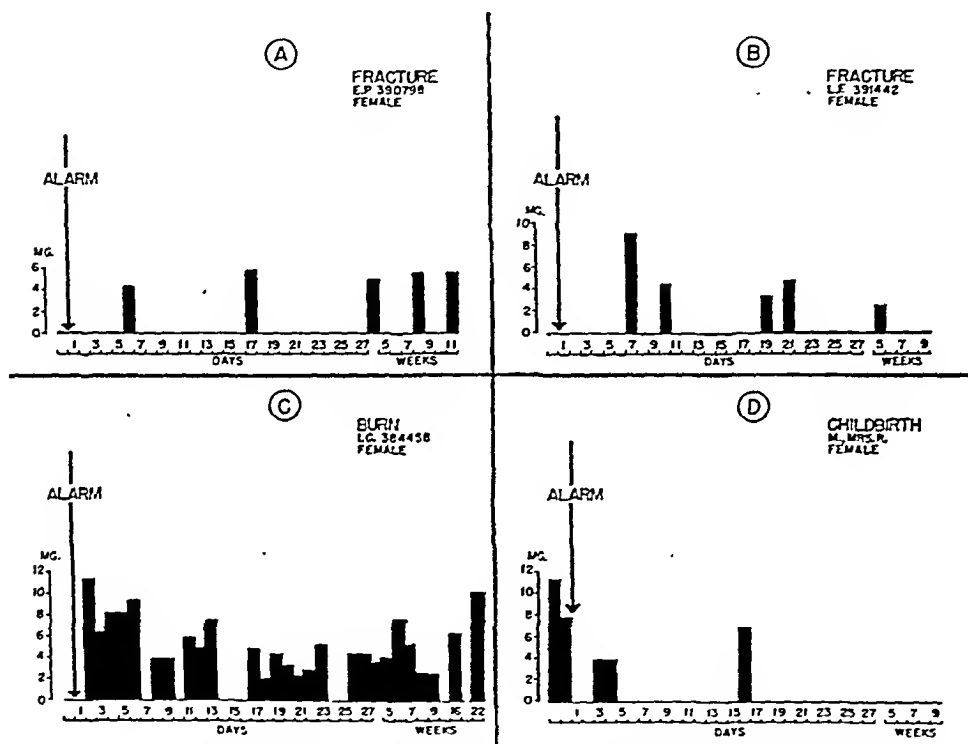


FIG. 11. The urinary 17-ketosteroid excretion of previously healthy women after fracture (A and B), burn (C), and childbirth (D).

cretion accompanying each malarial chill, but the peaks diminished progressively and the average level became lower and lower.

Figures 10A and 10B illustrate the effects of surgical operations on two healthy young women. The 17-ketosteroid curve was much the same as in males except that the peak which preceded the drop tended to be less pronounced. Figure 10C shows the excretion of another woman undergoing operation. This woman was obese and had hirsutism and irregular menses. She responded to operation with a peak of the same magnitude as the male cases in contrast to the women of Figure 10A and 10B. This may be characteristic of such women who usually have rather high 17-ketosteroid levels

normally (see also figure 12C). The point is of some interest since it supports the theory that we are measuring changes in the function of the adrenal glands and that changes in testicular function do not materially affect our results.

Figures 11A, and 11B show the excretion in cases of simple fractures in normal women; Figure 11C illustrates that of a severely burned woman.

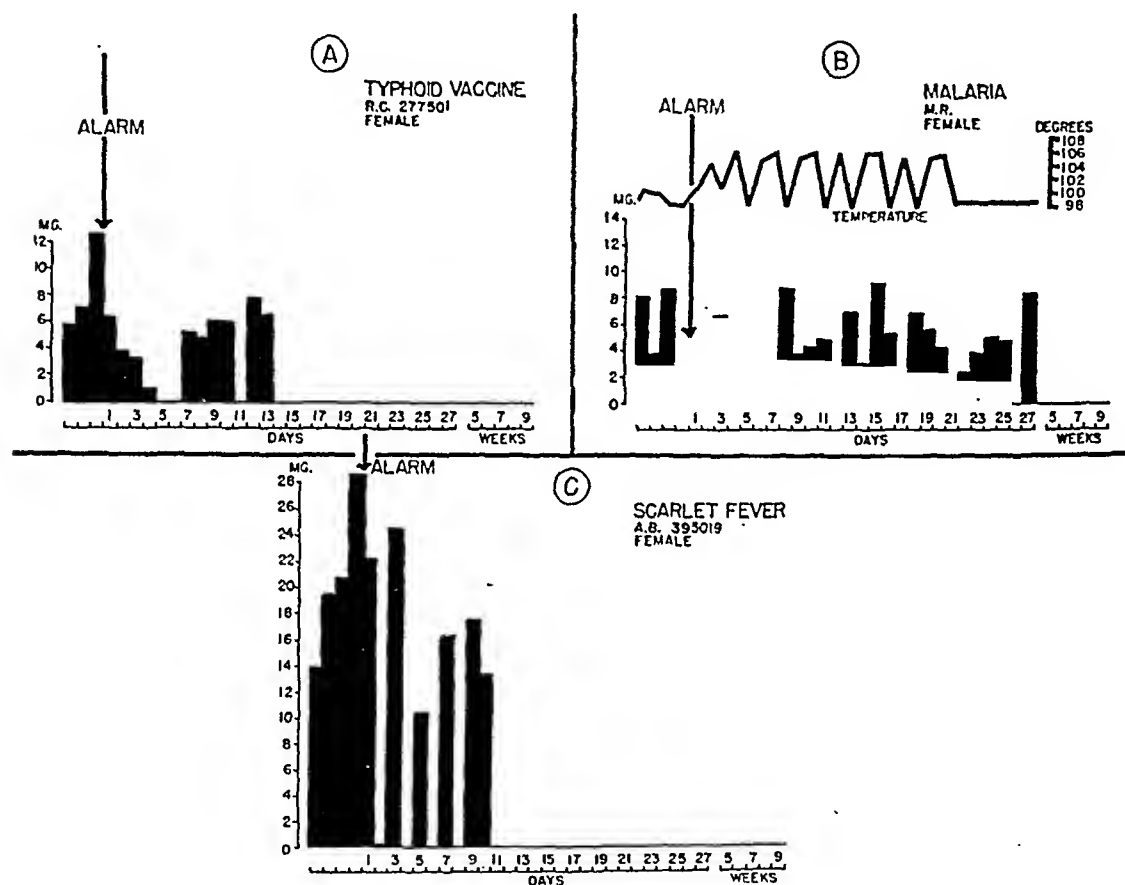


FIG. 12. The urinary 17-ketosteroid excretion of women with acute medical conditions: A—Fever due to typhoid vaccine, B—Malaria, and C—Scarlatinal wound infection in an obese, hirsute woman with irregular menses.

As in males, recovery following a severe burn was particularly prolonged. Figures 11D and 2B give the excretion of two women after normal child-birth. Here again there was a long recovery period, but in these cases other changes in endocrine status may have been playing a part.

The 17-ketosteroid excretion pattern of women with various medical conditions is illustrated by Figures 12A, 12B, and 12C. One of these, Figure 12 C, shows the reaction of an obese woman with hirsutism and irregular menses to a scarlatinal wound infection. Her normal excretion averaged 16.6 mg. per 24 hours which is not beyond the normal range, and

the infection caused a peak up to 28 mg. per 24 hours, which is decidedly high even for a male.

#### IV. The 17-Ketosteroid Excretion of Chronically Debilitated Patients After Acute Trauma.

We became interested in certain patients whose urinary ketosteroid excretion did not respond to injury with the pattern described above. The

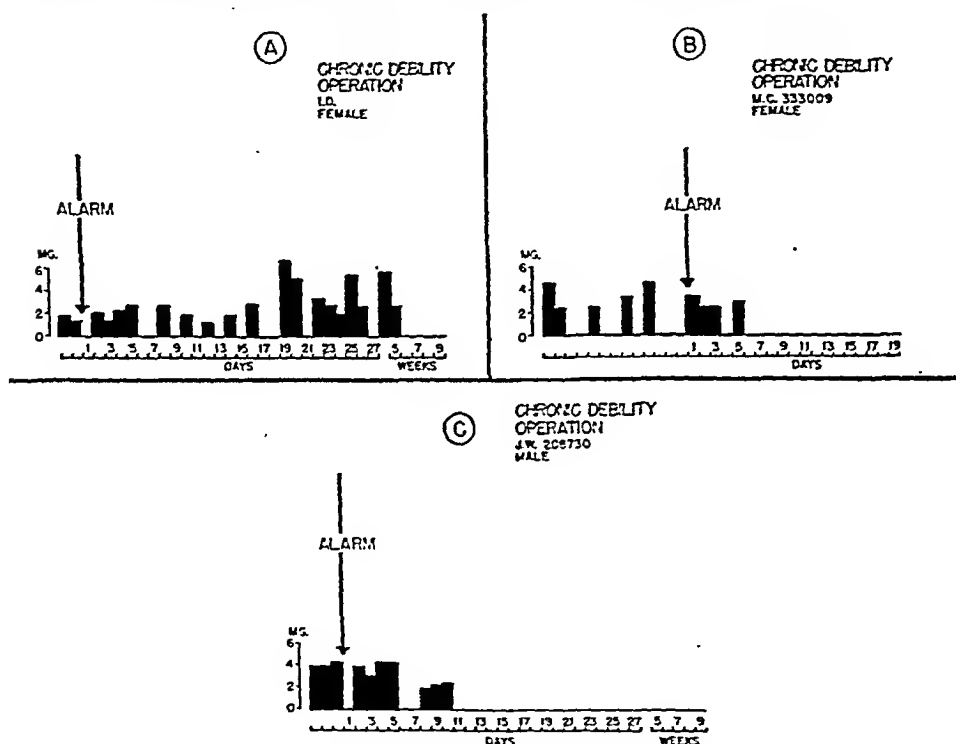


FIG. 13. The effect of acute trauma on the 17-ketosteroid excretion of three chronically debilitated patients: A. Woman with cerebro-spinal syphilis receiving malaria.\* B. Woman with hypertension and congestive failure undergoing splanchnicotomy. C. Man with empyema undergoing surgical drainage.

\* Incorrectly labelled operation.

first such patient whom we encountered (Fig. 13A) was a 43-year-old woman who received malarial blood for the treatment of cerebro-spinal syphilis. Her 17-ketosteroid excretion before treatment averaged 1.5 mg. per 24 hours. There was no peak and no significant fall after treatment although her temperature chart was similar to that of the other patients (see Fig. 12B). The second patient in this group, a 41-year-old colored woman with severe hypertension and cardiac failure, had an initial 17-ketosteroid level of 3.5 mg. per 24 hours before she underwent a sympathectomy.

There was neither a rise nor a significant fall (see Fig. 13B). A male patient who failed to respond is illustrated in Fig. 13C. He was a 30-year-old man admitted for drainage of an empyema with which he had been seriously ill for eight days before operation, who was in poor condition both before and after this procedure. The initial level of this patient was 4.0 mg. per 24 hours, which is a subnormal value. From these and other studies, we

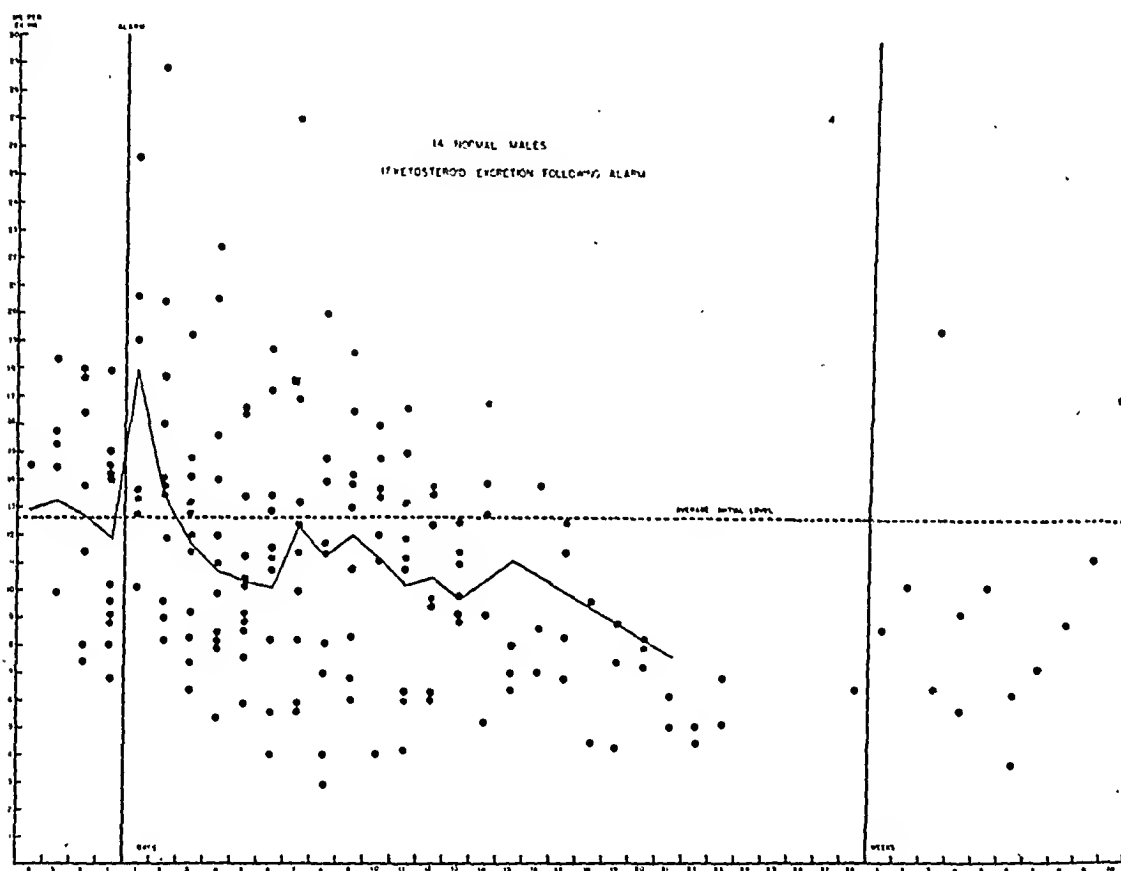


FIG. 14. Summary of the urinary 17-ketosteroid excretion of previously healthy men after acute trauma.

think that in those people who do not respond, the excretion level has already been lowered by chronic stress.

Figures 14 through 18 summarize the reactions of four different kinds of patients to injury. All of our cases both medical and surgical have been divided into four groups: (1) normal males; (2) normal females; (3) debilitated males; and (4) debilitated females. Patients of all sorts and ages have been included and the scatter is very great. The individual 17-ketosteroid values have been plotted against the number of days following the injury, and heavy lines have been drawn through the mean value of all of the patients on each day. These lines summarize our observations on the

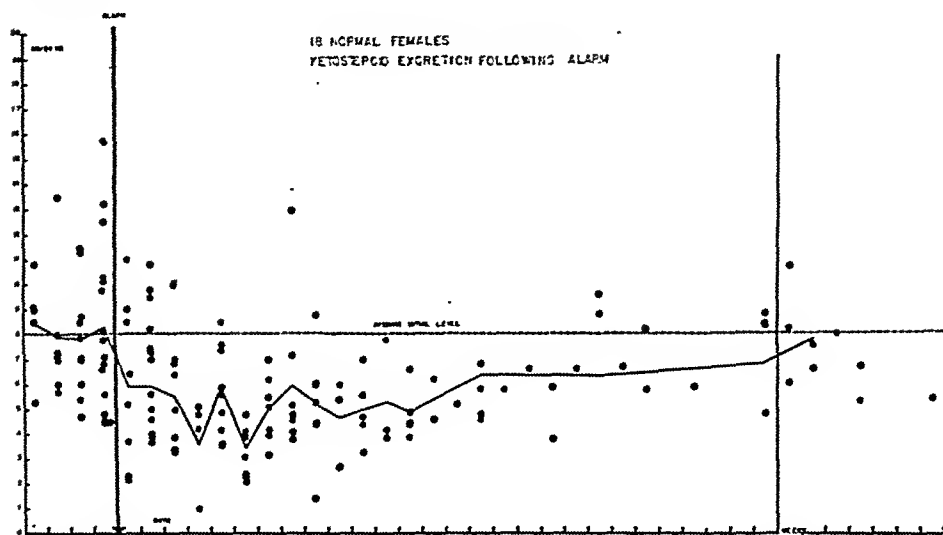


FIG. 15. Summary of the urinary 17-ketosteroid excretion of previously healthy women after acute trauma.

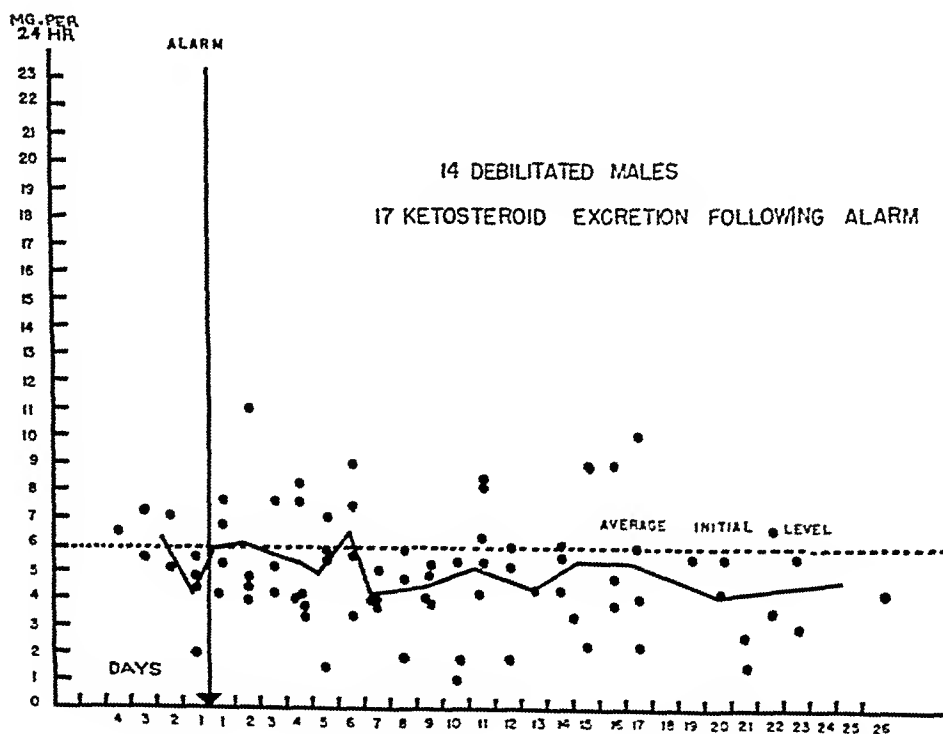


FIG. 16. Summary of the urinary 17-ketosteroid excretion of debilitated men after acute trauma.

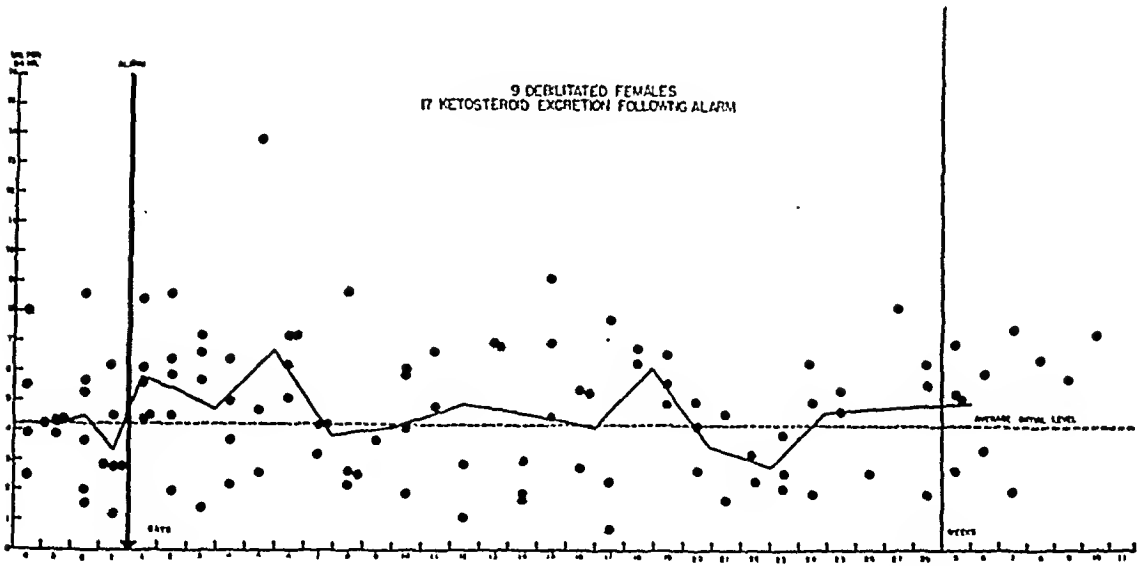


FIG. 17. Summary of the urinary 17-ketosteroid excretion of debilitated women after acute trauma.

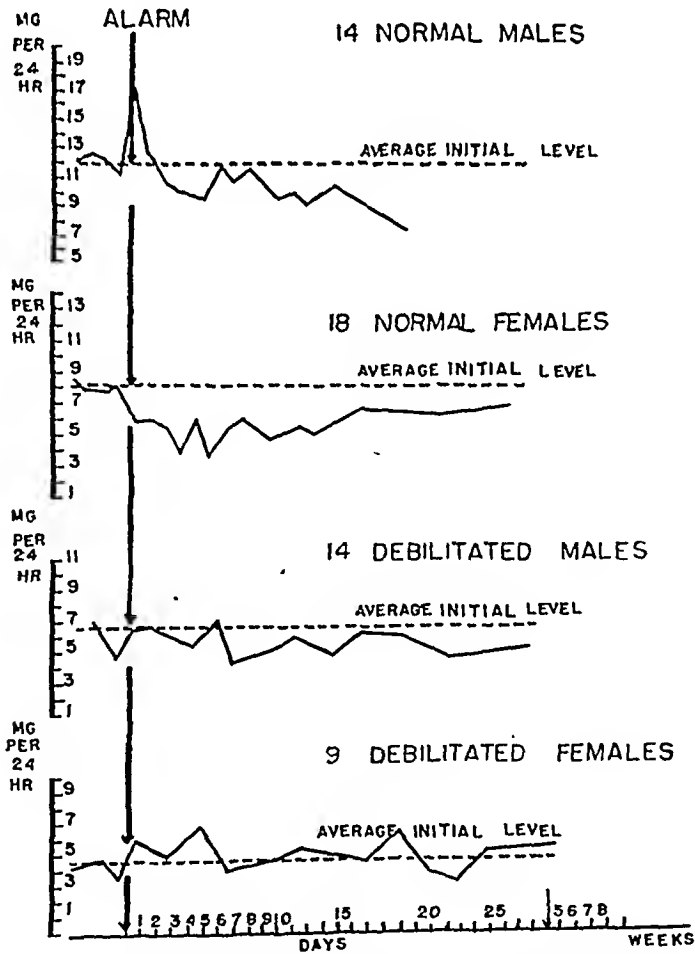


FIG. 18. The urinary 17-ketosteroid response of normal and debilitated men and women to acute trauma.

effect of trauma and disease on the urinary 17-ketosteroid excretion. They show: (A) in normal male individuals that the urinary 17-ketosteroid level responds to all kinds of bodily stress by a brief peak followed by a pronounced depression and a gradual return to normal; (B) in normal females that the response is the same but the peak is less pronounced; and (C) in chronically ill or debilitated individuals of either sex that the initial level is subnormal and the response to injury small or absent.

### DISCUSSION

The interpretation of our findings and their physiological basis has already been presented elsewhere (Albright (1)).

Selye has studied in animals the train of events which follows an injury regardless of the nature of the injury; this he terms the "Adaptation Syndrome" (25, 26). He divides the syndrome into three parts: (1) stage of alarm; (2) stage of resistance, and (3) stage of exhaustion. He further divides the stage of alarm into two parts, shock and countershock. In clinical medicine it is not quite clear where one stage ends and another begins and there has been a tendency to use the term "Alarm Reaction" as synonymous with "Adaptation Syndrome."

Many of the most striking features of the adaptation syndrome, Selye found to be secondary to alterations in adrenal cortical function. This was confirmed for humans by Browne (6).

In the adaptation syndrome it seems probable that at least two types of adrenal cortical steroids are involved, viz the "N" or nitrogen type of hormone and the "S" or sugar type of hormone.

The "N" hormone is not produced until puberty; it governs the growth of axillary hair in women; it has an anabolic action on protoplasm similar to testosterone; and it is responsible entirely in women and partially in men for the 17-ketosteroids excreted in the urine.

The "S" hormone, besides causing atrophy of the thymus and lymphoid tissue, has a profound but not clearly understood effect upon carbohydrate, protein and fat metabolisms. It unquestionably antagonizes the action of insulin. Many authors have been satisfied to summarize its metabolic effect by the assertion that it facilitates the conversion of protein into sugar (gluconeogenesis).

From studies here presented it appears that following a noxious stimulus of any kind there may be a temporary release of "N" hormone from the adrenal as judged by the rise in 17-ketosteroid excretion; this is followed by a decrease to subnormal levels which lasts until convalescence is nearly completed. The "S" hormone production on the other hand, as judged by the excretion in the urine (biological assay (Venning and Browne (32)) and chemical assay of 11-oxysteroids (Talbot, Salzman, Wixom and Wolfe



(31)), rises with the "N" hormone but remains elevated until convalescence is practically completed (Venning and Browne (32)). The early phase in which both "N" and "S" hormone are released is probably analogous to the end of the countershock phase in animals when the adrenal cortices histologically show depletion of lipid granules. The prolonged phase of low 17-ketosteroid excretion, coupled with continuous high "S" hormone excretion, probably corresponds to the phase of resistance. In those debilitated patients where an alarm is not followed by a rise in 17-ketosteroids it is probable that, due to a previous alarm, they are already in the stage of resistance or even approaching the stage of exhaustion.

The authors do not want to give the impression that the "N" and "S" hormones are the only adrenal hormones involved in the adaptation syndrome. Selye has indirect evidence that there is an associated increased excretion of the potassium-diuretic, sodium-conserving steroids similar to desoxycorticosterone. Smith and Smith (27) found a five to eightfold increase in the estrogen excretion of two postmenopausal and one surgically castrated woman following surgical operation.

#### SUMMARY

The urinary 17-ketosteroid excretion of sixty-six persons has been followed through some sort of medical or surgical injury. In normal individuals the urinary 17-ketosteroid excretion usually rises for a brief period (one to three days) and almost always falls for a longer period which lasts until convalescence is achieved when it gradually returns to normal. In chronically ill or debilitated individuals on the other hand, the initial level is subnormal and the response to injury usually small or absent. It is believed that these findings are further evidence that alterations in adrenal cortical function are an integral part of the adaptation syndrome of Selye and a link in our understanding of the part this syndrome plays in clinical medicine.

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# THE DIAGNOSIS OF HYDATIDIFORM MOLE BY GONADOTROPIC HORMONE ASSAY USING THE SOUTH AFRICAN FROG, *XENOPUS LAEVIS*\*

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SOON after Zondek and Aschheim made their brilliant discovery of the mouse test for pregnancy (1), Shapiro and Zwarenstein (5), Hogben (3), and Bellerby (2) showed the clinical application of the South African Frog (*Xenopus laevis*) to the finding of gonadotropic hormones in the urine of pregnancy.

In its natural habitat, the female of this unique species of amphibia never lays eggs unless stimulated by the male. Shapiro and Zwarenstein (6) segregated the females from the males and found that ovulation and external egg deposition could be induced entirely at will by simply injecting concentrates of pregnancy urine into these ever-ready and ever-willing female *Xenopus*. And thus, the early foundation for the frog test for pregnancy was laid.

Since 1939, we in America have studied *Xenopus laevis* in detail and have performed thousands of pregnancy tests with the animal (7, 8, 9, 10, 11).<sup>1</sup> In our earlier papers we have reported our excellent results, and have registered our keen delight with *Xenopus* as a test animal for pregnancy. In 1944, we showed the African Frog to be invaluable as an indicator of pregnancy owing to its clear-cut end reaction (8). There is no such report as "doubtful." Eggs are either laid or they are not. The reaction can occur as early as four hours after injection but is usually demonstrated in between six and twelve hours. The test is inexpensive since the animal can be used over and over again. The end-result is simple; the eggs are macroscopic; they resemble caviar and can be seen easily even by the most inexperienced eye. In our hands, the frog test was shown to be ninety-nine per cent accurate in pregnancy diagnosis (11).

In recent years, the response of *Xenopus laevis* to excessive amounts of

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<sup>1</sup> For purposes of collecting in one source book all the available data on *Xenopus laevis*, the authors were fortunate in obtaining the assistance of the New York Biologic Research Foundation in 1944 in publishing their monograph entitled "The South African Frog (*Xenopus laevis*) in Pregnancy Diagnosis." This monograph contains 262 references on *Xenopus laevis* in which are included 30 references to work performed with *Xenopus* in America.

chorionic gonadotropin has been of use in diagnostic laboratory procedures other than pregnancy diagnosis. Thus, in 1944 Zwarenstein and Duncan (13), and Weisman and Coates (12) demonstrated the use of the animal in the diagnosis of hydatidiform mole; and in 1946 Meiring and Zwarenstein (4) described the diagnostic value of *Xenopus* in certain types of testicular tumors.

At this time, we are reporting in detail the use of *Xenopus laevis* in differentiating pregnancy from chorionic pregnancy tumors (hydatidiform mole). As is well known, these chorionic tumors are usually associated with tremendous outpourings of gonadotropic hormones in the urine. In most instances<sup>2</sup> the hormone found in the urine of a patient suffering with such a tumor is as much as a hundred or even a thousand times as great as is found in normal pregnancy. Occasionally, an investigator will find a low titre of hormone in a case of hydatidiform mole but this is the exception rather than the rule.

*Xenopus* has been of great help to us in facilitating the differential diagnosis between pregnancy and the tumors of pregnancy. Such a differential laboratory test is of great value when clinicians are presented with a patient, thought to be pregnant, but whose abdomen has grown out of proportion to the slow growth of a normal pregnancy, and who has begun to stain vaginally. In our pregnancy work with *Xenopus* (12), it required one cubic centimeter or more of urine (usually concentrates are required) injected under the skin to evoke the ordinary pregnancy reaction. Urine of pregnant women diluted 1:10 with water never contained enough hormone to cause a reaction. On the other hand, in our experience urine in a dilution of 1:10 from patients afflicted with hydatidiform tumors was consistently found to induce ovulation in *Xenopus*. In some cases a dilution of 1:100 induced strong reactions. This quantitative difference in hormone excretion led us to suggest the following quantitative test in preference to the much slower mouse test previously utilized in the differential diagnosis between early pregnancy and tumors of pregnancy.

#### TECHNIC

The comparatively simple method of assay follows. Two female *Xenopus* are placed in each of three small fish tanks which are one third full of water. The animals in the first tank, A, are each injected with one cubic centimeter of the undiluted suspected urine; those in tank B are injected

<sup>2</sup> From time to time, there will appear in the literature a report of a normal pregnancy which by hormone titre approaches the enormous amounts excreted in chorionic tumors. Clinicians must realize that any laboratory diagnosis is merely an adjunct to their clinical impressions, and that in the final analysis the results of hormone assays can only be used to confirm or deny the clinical diagnosis.

with one cubic centimeter of a 1:10 aqueous dilution of the urine; those in the third tank, C, are injected with one cubic centimeter of a 1:100 dilution. After four hours the tanks are inspected at regular intervals for the presence of ova.

If, after twelve hours, the animals in tank A (whole urine) react by extrusion and there is no reaction in tanks B and C, the diagnosis of simple pregnancy can be made with accuracy. However, if in addition to the reaction found in A, the animals in tank B (1:10 dilution) are found to be depositing eggs, the probability of a pregnancy tumor cannot be dismissed. If animals in all three tanks are found extruding ova, the clinical presence of a far-advanced chorionic tumor cannot be disputed. In this latter instance, the amount of hormone is in excess of 100,000 *Xenopus* units per litre of urine. No other condition in the differential diagnosis aside from chorionic tumor of pregnancy (either hydatidiform mole or chorionepithelioma) will evoke so strong a response.

### RESULTS

Thus far, we have performed some thirty-one tests to differentiate pregnancy from hydatidiform mole. A tabulation of the results follows:

Reaction	Diagnosis
4 cases caused egg extrusion in all dilutions	—chorionic tumors
2 cases caused egg extrusion in 1:10 dilution	—chorionic tumors
25 cases evoked no response with undiluted urines	—no evidence of chorionic tumor
Total 31	

Subsequently, the laboratory diagnoses were all substantiated clinically. The six cases which caused egg extrusion in any of the dilutions were found to be hydatidiform moles; of the twenty-five suspicious cases one was found at laparotomy to be a large ovarian cyst and the remaining twenty-four were definite pregnancies.

### SUMMARY

A simple and rapid six to twelve hour method of assaying gonadotropic hormones in urine, using the South African Frog (*Xenopus laevis*), is presented.

Owing to the excessive amounts of this hormone found in the urine of patients suffering with hydatidiform moles (chorionic tumors of pregnancy) a dilution method of assay can be used successfully to differentiate these tumors from normal pregnancy.

Six cases out of thirty-one suspicious cases tested were successfully diagnosed preoperatively by this dilution method.

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# MALE HYPOGONADISM TREATED BY SUBLINGUAL METHYLTESTOSTERONE

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THERE have been only a few reports on the clinical administration of methyltestosterone sublingually for treatment of male hypogonadism. One of the earliest was that of Spence, who reported a single case (9). Another concerned twelve male hypogonads ranging in age from 19 to 73 years (6). In all, satisfactory development of secondary sexual characteristics took place and was maintained on a lesser dosage schedule using sublingual methyltestosterone.\* A subsequent report has confirmed the observations of others in regard to the effectiveness of relatively small doses of methyltestosterone sublingually (2).

Methyltestosterone sublingually may be administered in the form of Linguets or in a solution of propylene glycol. The former, however, is more practical than the solution, since solid material can be placed between the buccal mucosa and the tongue. In this locality, the Linguet usually remains unnoticed during the period of absorption, causing no inconvenience or restrictions beyond the admonition to refrain from "tonguing" the wafer and a temporary ban on eating during the time the Linguet remains in the mouth.

In previous reports the author has indicated the effectiveness of oral methyltestosterone in the treatment of male hypogonadism, (2, 3, 4). Since the advent of the Linguet form, a number of patients have been placed on this therapy, and the following case histories are typical of the response that can be expected. Sublingual administration of methyltestosterone ranged from 10 to 30 mg. daily in divided doses.

*Case 1.* C. E., age 36, married 8½ years, older brother of patient number 2. One uncle and two brothers are eunuchoids. The patient had a long history of cardiac disease and had been treated for cardiac dysfunction before coming to the clinic and being placed on testosterone therapy. It is of interest to note that cardiovascular imbalance has been reported to be frequently associated with hypogonadal types (8, 7).

Physical examination revealed bilateral cryptorchidism, extreme genital infantilism and chronic endocarditis. The patient's voice was markedly feminine. Previous therapy with testosterone had been irregular and insufficient.

Methyltestosterone, 30 mg. per day in the form of Linguets, was prescribed and this therapy continued for a period of five months. Within the first three weeks there was

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\* The methyltestosterone tablets in the form of Linguets (Metandren) were supplied by the courtesy of Mr. Charles E. Munson of Ciba Pharmaceutical Co., Summit, N. J.



definite genital development (Fig. 1), and at the end of six weeks there was obvious deepening of the voice. Between the third and fourth weeks it was noted that both testes were beginning to descend. The patient reported marked improvement of vitality, and his endurance was considerably enhanced.

Although various reports have indicated that androgens produce favorable metabolic effects and that some of this may be on the basis of cardiotropic action, it is a well known fact that androgenic therapy may produce augmentation of nervous and muscular



FIG. 1. C. E., aged 36, (*Case 1*). Improvement following two and a half months of therapy with Linguets.

activities which may be at variance with the moderation indicated by the condition of the heart and blood vessels, (5).

With the increased physical activity of this patient, his cardiac disorder became more marked; he developed a double murmur with partial decompensation and had to be confined to bed. It was deemed advisable to discontinue androgenic therapy because of the excessive stimulation in the physical sphere.

*Case 2.* J. E., age 28, married 4 years (see family history of older brother above). Physical examination revealed marked hypogenitalism, absence of facial hair, and scanty pubic and axillary hair. The patient talked in a very high-pitched voice. He complained of low energy and vitality. Previous therapy, which had been irregular and in-

sufficient, had consisted of intramuscular injections with testosterone propionate and chorionic gonadotropin, as well as pregnant mare serum. He had reacted well to testosterone injections with more frequent erections and a somewhat increased vitality, which, however, lasted only during the period of therapy. Other forms of treatment had produced no positive results. Linguets were started in a dosage of 15 mg. per day and continued for a month. At this time the dosage was decreased to 10 mg. per day, because the patient noted scrotal congestion and pain, whereas 1 Linguet (5 mg.) daily



FIG. 2. J. E., age 28, (Case 2). Two and a half months of treatment with Linguets.  
J. E. is younger brother of C. E. (Case 1).

was not sufficient to cause any positive response. At the end of 17½ months the patient discontinued treatment of his own volition as he thought that it "increased his heart rate."

During the period the patient was being treated, there was noted a definite increase in the size of the genitalia (Fig. 2); there were an increased number of erections and obvious deepening of the voice. Pubic and axillary hair began to increase and there appeared a small amount of hair on the face and chest. At the end of 17½ months of therapy muscular development had increased to the extent that the patient required shirt size 15½, whereas before he required only 14½.

One year after discontinuing treatment, the patient showed some regression. During

treatment a small cystic mass, about the size of a cherry, appeared in the lower pole of the right testicle. This, however, gradually decreased and disappeared on cessation of therapy.

*Case 3.* J. G., age 21½, single. Physical examination revealed marked genital infantilism. This patient had been treated with pregnant mare serum for a period of about 2 months with no results whatsoever. He was then treated with testosterone propionate injections for a period of 1 month, totaling 250 mg. During this time, there was an increase in general muscular development, an increase of pubic hair, a deepening of the voice and gain in weight and vitality. Following this improvement, testosterone pellets totaling 300 mg. were implanted. One week later one pellet was expelled and later another pellet expelled. Two months later a second implantation of 300 mg. of testosterone pellets was carried out. Three months later he required injections of 25 mg. of testosterone propionate per week and these were continued for a period of about eight months, totaling 1223 mg. During the next nine months the patient received no therapy, but because of a recurrence of his symptoms oral methyltestosterone 20 mg. per day was prescribed and injections of 25 mg. of testosterone propionate were administered twice a week. There was a further increase in the size of the genitalia, accompanied by complaints of gynecomastia and three to four erections per day.

The patient was placed on Linguets, 15 mg. daily. It was found that this dosage caused too many erections and it was, therefore, decreased to 10 mg. per day. Linguets were used exclusively for economic reasons inasmuch as they are about half the cost of pellets.

On Linguet therapy the patient continued to improve and there was development of facial hair. The gynecomastia regressed. Excessive physical exercise was forbidden because of the detection of a faint heart murmur.

*Case 4.* L. M., age 30½ years, married 6 years. Physical examination revealed bilateral cryptorchidism, genital infantilism and absence of facial or body hair.

He had been treated with chorionic gonadotropin, thyroid, androsterone and testosterone, with only slight or moderate improvement. Testosterone therapy in 1936 had apparently caused some increase in the size of the genitalia and a moderate deepening of the voice. In October, 1944, testosterone pellets totaling 525 mg. were implanted. A re-implantation was done five months later with 300 mg. of testosterone and at four and five months, respectively, subsequent implantations of 300 mg. each. There was marked improvement in both the physical and sexual spheres following each implantation of testosterone pellets.

The patient was placed on Linguets, 15 mg. per day, and this dosage was maintained for one and one-half years. Remarkable improvement in the growth of the genitalia was noted (Fig. 3). There was rapid progress in regard to penile erections and emissions, as well as a rapid growth of body musculature and physical development. Facial hair and facial expression changed from a juvenile to a stern, masculine demeanor. When the patient took 4 Linguets per day, tachycardia was noted, and when the dosage was decreased to 2 Linguets per day, it proved insufficient to maintain progress. Cryptorchidism was not influenced by any therapy, though the presence of a small, soft body in the mid-portion of the right inguinal canal was noted occasionally. The patient changed to implantation treatment because of convenience only.

*Case 5.* E. P., age 43, single. This patient complained of extreme nervousness, inability to sleep, lack of concentration, occasional hot flashes, and frequent headaches. Physical examination revealed a rather obese individual, with evidence of surgical castration. History revealed that tuberculosis of both testes had necessitated their surgical removal

at the age of 20. Castration symptoms were brought under control with testosterone propionate injections in 25 mg. doses two to three times per week, and later with tablets of methyltestosterone 10 to 20 mg. per day. He was then placed on methyltestosterone Linguets 20 mg. per day; this was subsequently reduced to 10 mg. per day and therapy continued for two years with satisfactory results.

During this two year period a nephrectomy was performed for tuberculosis of the right kidney. Temporary withdrawal of the Linguet therapy resulted in a recurrence of

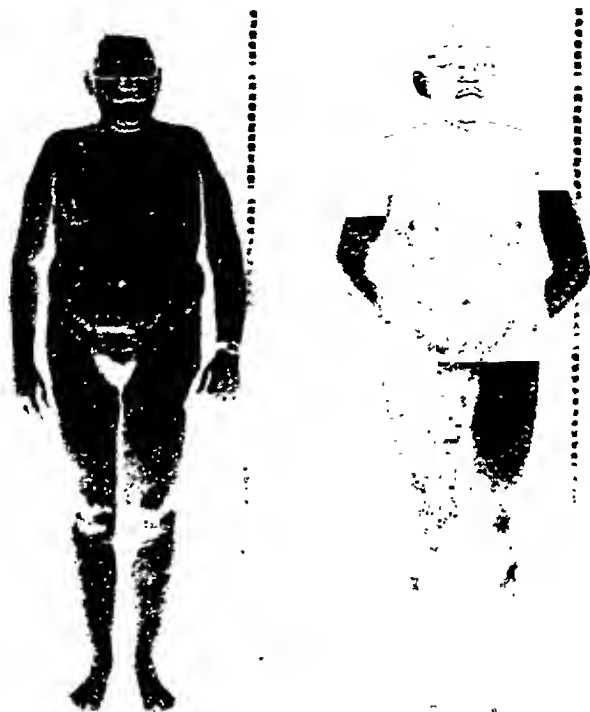


FIG. 3. L. M., age 30½, (Case 4). Originally treated with chorionic gonadotropin, thyroid, androsterone and testosterone with slight improvement. Pellet implantations produced marked improvement in physical and sexual spheres. Progress maintained and additional improvement on Linguets, 15 mg. per day.

symptoms, and the patient insisted on permission to resume the therapy a few days postoperatively.

There was general improvement in all of his symptoms on Linguet therapy; fatigue, cramps in the legs and arms disappeared. There was, however, no sexual stimulation as had been observed when taking methyltestosterone orally in doses of 10 mg. per day. Headache had recurred occasionally but there were no hot flashes; concentration was good. He found that the effect of 1 Linguet lasted from four to five hours.

Case 6. H. R., age 29, single. Physical examination revealed definite hypogenitalis,

absence of facial and body hair and a high-pitched voice. Previous therapy had consisted of a number of injections of testosterone propionate and repeated implantations of testosterone pellets. Initial priapism had been produced following the injection of testosterone propionate, 25 mg. three times a week. This was reported previously, (1).

Following implantation therapy, the patient experienced normal erections, normal libido, and was able to carry out normal sexual relations. In September 1944, the patient was given Linguets, 15 mg. per day, and continued on this therapy for a period of 7 months, during which time excellent results were reported, and although he could be maintained on a slightly decreased daily dosage of 10 mg., he seemed to do better on 15 mg. per day.

*Case 7.* M: Y., age 31, single. Physical examination revealed right cryptorchidism, genital infantilism, absence of body and facial hair, a moderately high-pitched voice and excessive girdle obesity and lack of energy and endurance. He expressed a feeling of insecurity and confided that he contemplated marriage, and therefore wished to achieve a fair degree of sexual development. There had been no previous therapy.

Linguets, 15 mg. per day, were prescribed in August 1943, and this dosage was increased to 20 mg. per day at the end of about ten days. Therapy on this schedule was maintained until December 1943. During the next three months a total of 750 mg. of testosterone propionate was administered in 25 mg. doses three to four times per week, because the patient was anxious to speed up the rate of development. This method of therapy did not seem to result in further increase in the size of the genitalia; it merely maintained the results already achieved.

While on Linguet therapy, there was a definite increase in the size of the left testicle the right testicle enlarged somewhat, but remained in the inguinal canal, and there was a gradual but definite increase in the size of the penis (Fig. 4), a deepening of the voice, an increase of pubic and axillary hair and some loss of adiposity. There was a definite increase in physical strength and endurance as well as in mental outlook and social adjustment. As the patient stated himself, he lost his shyness and feeling of inadequacy and acquired new interests in people and events.

Implantation of 300 mg. of testosterone was made in April 1944, in order to avoid the need of constant oral therapy, and the patient was married in June 1944, and able to carry on normal sexual relations. An additional implantation was made in September 1944, and a further implantation in February 1945. There was no further progress following implantation therapy, although the initial progress was well maintained. In July 1945, half of a pellet was expelled and in August another pellet was expelled. Pellets continued to be expelled, and because of this, Linguet therapy was resumed in December 1945. The original prescription of 15 mg. per day maintained the initial progress but produced no further increase in the development of the genitalia.

*Case 8.* E. B., age 21, was first seen on August 8, 1946, because of obesity and an arrest in genital development.

He was operated on at the age of 13 for bilateral cryptorchidism and an inguinal hernia. Prior to the operation, the patient was treated with Antuitrin "S" for two years. At the time of the operation, an atrophic left testicle was found in the inguinal canal and was placed in the scrotum; the right testicle was fairly well developed and was also brought down from the inguinal canal into the scrotum.

The patient began to gain weight steadily and in 1942, four years after the operation, he weighed 173 lbs.; there was no evidence of either testicle in the scrotum or the inguinal canal. The patient exhibited characteristic features of a eunuchoid, such as absence of

facial and body hair, scant pubic hair with female distribution, and lack of energy.

He was advised to begin glandular therapy, but he did not return to his physician until four years later (eight years following the operation) and was then advised to see an endocrinologist.

On initial examination, his height was 69½ inches, weight 206 lbs., pulse 80, blood pressure 112/60, heart and lungs normal. The body configuration was of eunuchoid proportions; there was an absence of facial and body hair, and the pubic hair line was

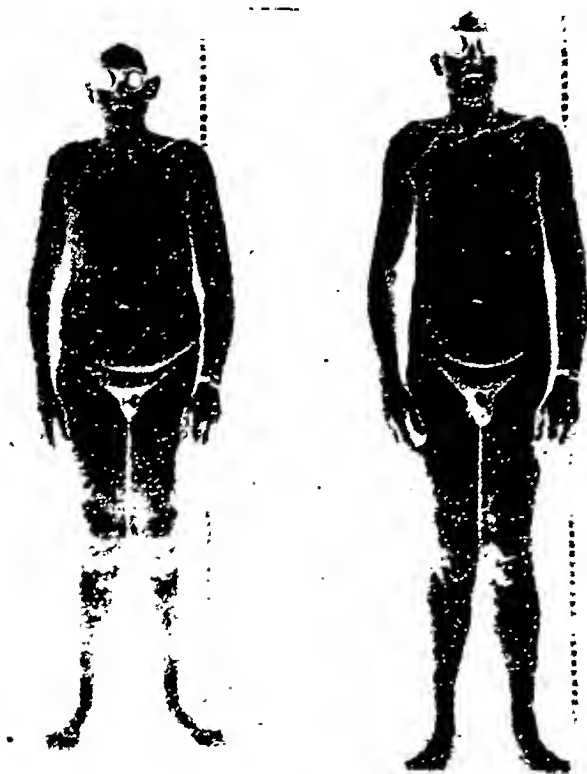


FIG. 4. M. Y., age 31, (Case 7). The above improvement obtained with Linguets, 20 mg. per day for four months. Patient subsequently treated with injections of testosterone propionate in an effort to speed up degree of development.

horizontal. The penis was abnormally small, serotum shrunk and there was no evidence of testes either in the serotum or in the inguinal canal. The voice was not high-pitched. There was complete lack of libido, erections and emissions.

The BMR was minus 22 and x-ray studies of the skull and sella turcica were within normal range. Roentgenological examination of the long bones showed failure of epiphyseal union of the radius and ulna.

The patient was placed on Linguets, 20 mg. per day, for six weeks, (this dosage was later reduced to 15 mg. per day), thyroid substance gr. 1 twice a day, a high protein, low fat and carbohydrate diet.

After four months of therapy, the patient showed a loss of 40 lbs., and an increase in energy and mental alertness, in growth of the external genitalia, and improvement in facial expression. (Fig. 5).

When last seen on October 22, 1946, the patient was very pleased with the progress

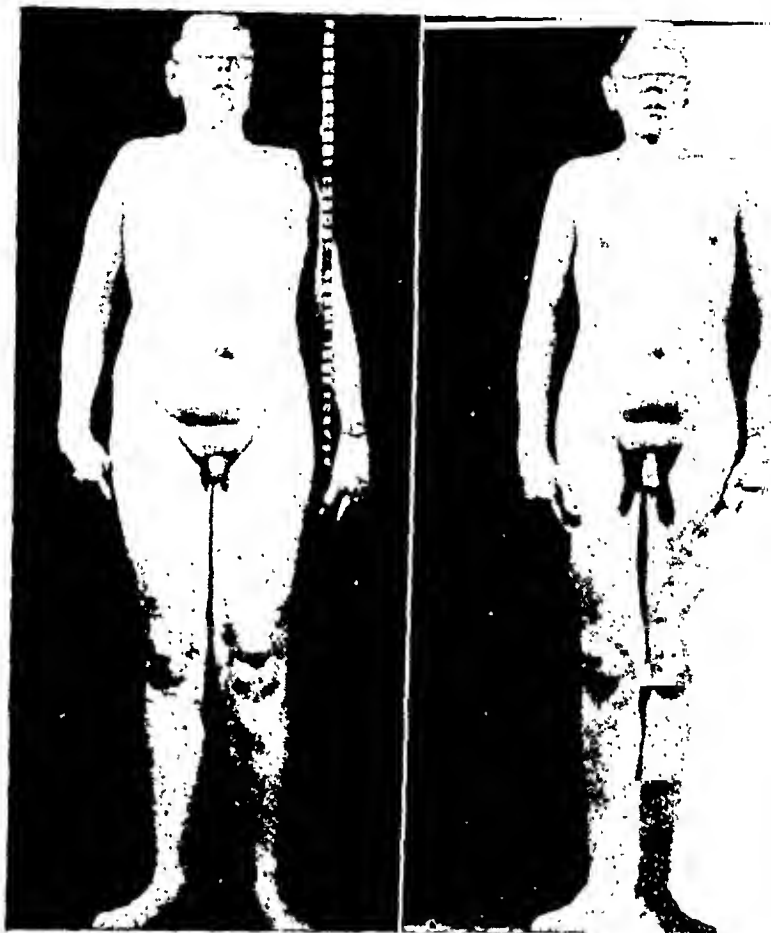


FIG. 5. E. B., age 21, (*Case 8*). Treated with Linguets and thyroid over a four-month period. Initially, 20 mg. of Linguets per day for six weeks and thyroid gr. 2 per day. Later, Linguets, 15 mg. per day. On this therapy the patient lost forty pounds in four months.

made and was eager to continue the therapy. The dosage of the Linguets was further decreased to 10 mg. per day, and the thyroid substance and diet continued.

The remaining four cases are briefly as follows:

*Case 9.* D. D., age 25, had been treated for hypogonadism, lack of energy, headaches and gynecomastia for the last five years with testosterone pellet implantations at periodic intervals. Because of his physical condition he was shy, reticent and did not mingle socially. He responded well to the testosterone therapy with an increase in energy, mental concentration, endurance and self assurance; however, he failed to develop facial hair to any extent. In order to achieve the latter, methyltestosterone Linguets 10 mg. daily were added to the implantation therapy. This resulted in a definite increase in facial hair. He was eventually placed on Linguet therapy exclusively, 15 mg. daily, with satisfactory maintenance.

*Case 10.* A. R., age 31, was treated with intensive chorionic gonadotropin therapy for genital and somatic infantilism, lack of energy, endurance and libido. A definite increase in the size of the genitalia occurred. There was great improvement in vitality, endurance and mental outlook. A moderate growth of hair appeared on the face and body. Because of the patient's desire to appear more masculine, he was placed on methyltestosterone Linguets 30 mg. per day; the original results achieved were maintained satisfactorily and the growth of facial and body hair was accelerated.

*Case 11.* L. Z., age 26, was treated with chorionic gonadotropin in large doses for cryptorchidism, hypogenitalism, obesity, debility, headaches and a high-pitched voice. He responded moderately well with a deepening of voice, increased energy, moderate increase in the growth of the genitalia and body hair. Since he still lacked endurance and was still subject to headaches, he was placed on methyltestosterone given sublingually 10 mg. daily, and responded favorably with an improvement in all the deficiencies mentioned above.

*Case 12.* W. D., age 22, a male pseudohermaphrodite, was treated intensively with testosterone in order to enhance the development of male secondary sex characteristics. The initial therapy consisted of injections with testosterone propionate and implantations with testosterone pellets. The therapy produced satisfactory results, but for the sake of convenience, he was placed on methyltestosterone Linguets, 20 to 30 mg. per day. This therapy maintained the original results achieved and caused further progress in muscular and genital development, deepening of the voice and an increase in energy and endurance.

#### SUMMARY

A group of 12 hypogonadal men (10 eunuchoids, 1 castrate, 1 male hermaphrodite) were treated with methyltestosterone tablets especially prepared for use by the sublingual route.

Each tablet contained 5 mg. of methyltestosterone; the daily requirement was from 2 to 6 tablets according to the needs of the patient.

The subjective and objective response of these patients was similar to that obtained with injections of testosterone propionate or implantations of testosterone pellets. This response can often be initiated or maintained on comparatively small dosage schedules.

In some of these patients the increased physical activity was at variance with the capabilities of the cardiovascular system, and the dosage was reduced accordingly; therapy was discontinued entirely in one case.

In all cases herein reported there was definite enlargement of the phallus and scrotum. A more male-like growth and distribution of body hair was observed. There was increased vigor, less tendency towards fatigue and a general personality change resulting in more masculine aggressiveness.

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even in the absence of the thyroid. However, adrenals, kidneys and heart appeared to be less sensitive to these trophic effects than in the presence of the thyroid.—*E.C.R., Jr.*

LUKENS, F. D. W. Pituitary-diabetes. *Am. J. Med. Sci.* 212: 229 (1946).

The author surveys the present state of affairs of pituitary-diabetes in terms of three aspects: 1) The identification of the hormones concerned with the metabolic activity of the pituitary; 2) the physiologic mechanism by which pituitary extracts, crude or refined, initiate the phenomena of diabetes; and 3) the relation of the pathogenesis of the permanent phase of diabetes with its obvious lesions of the islands of Langerhans to the preceding functional effects of the pituitary extract. Citation is made to 50 references. The author's summary follows. "According to studies done so far, no single purified pituitary hormone contains all the diabetogenic properties. Growth hormone is ketogenic and by the present limited criteria inhibits the utilization of carbohydrate. The adrenotrophic hormone mobilizes protein and carbohydrate and so is necessary for the phenomena of overproduction observed in diabetes. On theoretical grounds, maximum diabetogenic activity would result from a combination of these hormones, and this seems to be the case when crude extracts are employed. The mechanism of action of crude pituitary extracts is still poorly understood. However, this action includes evidence of the suppression of carbohydrate utilization and of overproduction under certain conditions. The effects of crude extract on glycogen storage, protein metabolism and fat metabolism compose a picture not incompatible with the gross observation that protein or carbohydrate food is necessary for the greatest diabetogenic effect. The suggestion has been made that the degree of glucose production and utilization needed by the fasting animal is so established or "fixed" by pituitary extract that carbohydrate or protein food cannot be metabolized in the normal manner and hence leads to hyperglycemia. If this hyperglycemia is sustained long enough, at least in the dog and cat, the islands of Langerhans undergo a sequence of structural changes resulting in irreversible damage and permanent diabetes."—*E.C.R., Jr.*

## THYROID

ASTWOOD, E. B., AND W. P. VANDERLAAN. Treatment of hyperthyroidism with propylthiouracil. *Ann. Int. Med.* 25: 813-821 (1946).

Propylthiouracil was administered to 100 patients with thyrotoxicosis during the course of one year. The minimal daily dose of the drug necessary to restore metabolic equilibrium varied from 50 to 150 mg. Except for four instances of transient pruritis and one instance of urticaria, no toxic reactions were observed. Five patients who had developed febrile reactions on thiouracil and two patients who developed fever on thio-barbital were able to take propylthiouracil without untoward effect.—*J.M.*

BARMAN J. M. AND E. PORCILE. A simple method for assuring accuracy in determination of oxygen consumption by the Benedict-Roth apparatus. *J. Lab. & Clin. Med.* 31(11): 1254-1256 (1946).

In performing tests of basal metabolism in many patients with an irregular respiratory rhythm, it is difficult to draw the "oxygen line" accurately. Indeed, any of two or three lines may appear equally correct. Attempt is made to draw such lines through points of the curve corresponding to the end of expiration, at which time the lung contains both reserve (supplemental) and residual air. The volume of reserve air varies with

the depth of respiration. The depth of respiration, while commonly uniform in normal subjects, often varies considerably in diseased states, such as hyperthyroidism, and thus creates the difficulty mentioned above. To obviate this, the authors propose that at three or more times during the metabolic test the patient be instructed to exhale maximally, thus removing all supplemental air. Under such circumstances, they have found that the three or more points corresponding to the end of maximal expiration are in a straight line, and that oxygen consumption calculated with such a line as a basis corresponds closely to that obtained by gas analysis according to the Tissot-Haldane open circuit method.—*T.H.McG.*

BEIERWALTES, W. H., AND C. C. STURGIS. Complications following the administration of thiouracil. *Am. J. Med. Sci.* 212: 513 (1946).

From their experience in treating 80 cases of toxic goiter with thiouracil, the authors conclude that thiouracil is a valuable drug when employed preoperatively in patients with both nodular and hyperplastic toxic goiter. It is superior to iodine when used in this way because the basal metabolic rate may be brought to zero or lower, and also because it is equally effective in both types of toxic goiter. Iodine has not been highly satisfactory in the preoperative treatment of patients with toxic adenoma or in many cases with exophthalmic goiter especially when it has been previously administered, or possibly when the patients have ingested iodized salt over a long period of time. Only 1 patient in the group of 80 failed to show a basal metabolic rate below zero after thiouracil medication. The authors collected from the literature and by personal communication 10 fatalities occurring during or following thiouracil administration; of these 7 were definitely attributable to agranulocytosis. Analysis of these cases revealed a common pattern which gives clues to follow in the prevention of fatalities due to this complication. The authors recommend that the patient who is taking thiouracil be warned to discontinue the drug promptly following the appearance of fever with or without sore throat, skin rash or adenopathy. If a white blood cell count and estimation of the neutrophil percentage shows a significant depression of the cells, penicillin should be administered immediately until such time as the blood count becomes normal again. By such procedure the authors were able to avoid fatality in the three episodes of agranulocytosis that developed in their series. When the blood count becomes normal, thiouracil therapy may be resumed, but it is recognized that there is a definite risk of recurrence of the agranulocytic reaction. Other complications ultimately requiring thyroidectomy are febrile reactions, relative thiouracil resistance, nodular thyroid, large goiter, and failure of the patient to cooperate. The remaining complications (dermatitis, edema of the legs, jaundice, enlargement of the thyroid, production of accentuation of exophthalmos) occurring during thiouracil administration ordinarily do not require more than temporary cessation of the drug.—*E.C.R., Jr.*

DOBYNS, B. M. Exophthalmos and tissue changes in the guinea pig following administration of the thyroid stimulating hormone of the pituitary gland. *West. J. Surg. Obst. and Gyn.* 54: 411-427 (1946).

Exophthalmos was produced in guinea pigs by the injection of thyroid stimulating hormone, regardless of the presence or absence of the thyroid or testicles. A generalized alteration in fat metabolism occurred throughout the body, together with a connective tissue reaction. Fat depots were depleted and replaced by edema and connective tissue. Large quantities of lipoid appeared in the liver, kidneys, muscles, and in some epithelial

and reticulo-endothelial cells. It is suggested that many of the changes in the orbital tissue which produce exophthalmos are expressions of generalized tissue changes.—*J.M*

DOBYNS, B. M. AND S. F. HAINES. Changes in the prominence of the eyes in various thyroid states. *J. Lab. & Clin. Med.* 31(4): 483-484 (1946).

Thyroidectomy in patients with exophthalmic goiter usually was followed by increased exophthalmos. If hypothyroidism occurred postoperatively, the change was more marked. Treatment of both spontaneous and postoperative hypothyroidism decreased the prominence of the eyeballs. Thiouracil given to patients with exophthalmic goiter caused an increase in exophthalmos commensurate with the drop in basal metabolic rate. In the majority of all the studies, the changes were of such low magnitude as to be perceived only by careful exophthalmometry.—*T.H.McG.*

GORDON, A. S., P. C. KADOW, G. FINKELSTEIN, AND H. A. CHARIPPER. The thyroid and blood regeneration in the rat. *Am. J. Med. Sci.* 212: 384 (1946).

From studies in rats, the authors concluded: 1) thyroidectomy or thiouracil treatment inhibited the return of the red blood cells and hemoglobin to normal levels following bleeding. Studies of the leukocyte changes were inconclusive. Injections of thyroxin speeded regeneration of the red blood cells and hemoglobin to a greater degree in bled thyroidectomized than in bled unoperated rats. It is still an open question as to whether the thyroid directly regulates the erythropoietic process or whether this control is a secondary manifestation of the changes in general metabolism induced by the gland. 2) Castration in thyroidectomized animals did not further delay red blood cell regeneration, but caused a greater inhibition of hemoglobin synthesis. Testosterone was a more effective erythropoietic agent in the thyroidectomized-castrated than in the thyroidectomized rat. The combination of testosterone and thyroxin was little more effective than thyroxin alone. The authors believe that testosterone affects the erythropoietic process itself. Cobalt markedly stimulated red blood cell and hemoglobin production in bled thyroidectomized rats; the combination of cobalt and thyroxin was even more powerful. It is suggested that small amounts of this metal be administered along with the appropriate hormones in the treatment of clinical anemias accompanying endocrine deficiency. The hormones and cobalt, alone or in combination, all evoked marked reticulocytosis during the week following bleeding; but cobalt, and cobalt in combination with thyroxin, were the only treatments which maintained the reticulocytes at high levels throughout the experiment. Neither thyroidectomy nor administration of hormones or cobalt caused any significant change in the total or differential white cell counts of bled rats. The bone marrow of thyroidectomized rats displayed a slightly greater state of hypoplasia and a lower erythroid/myeloid cell ratio than that seen in unoperated animals for 3 to 4 weeks after the bleeding. Thyroxin tended to correct these changes; but cobalt, and especially the combination of cobalt and thyroxin, produce the greatest degree of marrow hyperplasia.—*E.C.R., Jr.*

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## POLYOSTOTIC FIBROUS DYSPLASIA: A DEFENSE OF THE ENTITY

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**I**N 1937 and 1938 the author and his colleagues published two papers (1, 2) in which they described the clinical findings of 7 patients with a bizarre syndrome characterized by "osteitis fibrosa disseminata, areas of pigmentation, and precocious puberty in females." Since that time some authors (3, 4, 5, 6) have been so flattering to the present author as to publish similar cases under the heading of "Albright's Syndrome"; others (7, 8) have questioned the entity of the syndrome. It is the purpose of this paper to defend the entity; a few words pertaining to terminology will be added.

For the time being let us call the condition under discussion, "Syndrome X."

### Is Syndrome X a Form of Lipoid Granulomatosis?

Snapper (7) feels that Syndrome X is a form of Hand-Schüller-Christian's Disease (xanthomatosis) which he prefers to call "lipoid granulomatosis" and which is closely akin to, if not identical with, eosinophilic granulomatosis. He points out that, whereas the classical case of lipoid granulomatosis is characterized by multiple round defects in the skull, exophthalmos, diabetes insipidus, and other pituitary signs, there need be no cranial or hypophyseal localization; he states that it is in such cases that the condition "is usually not recognized and goes under a different

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name." At this point he cites the cases described by the author. To be sure, in the summary he is less dogmatic when he states that "it seems reasonable to conclude that at least part of the cases of osteitis fibrosa disseminata . . . may well belong to the group of lipoid granulomatosis of the bones without cranial hypophyseal localization. . . ."

Snapper leaves the reader with the impression that the author and his colleagues separated the condition under discussion from lipoid granulomatosis for two reasons only, the lack of hypercholesterolemia and the failure of bone biopsies to show any tissue abnormalities other than osteitis fibrosa. As a matter of fact these two arguments, while definitely of some importance, bear less weight than several others, one of which was emphasized in the original article.

The arguments against Syndrome X being a form of lipoid granulomatosis will now be discussed one by one.

The finding of a normal blood cholesterol level does not rule out a disorder of cholesterol metabolism, but it is perhaps some evidence in that direction; however, it is certainly consistent with the findings in many cases of undoubted lipoid granulomatosis.

Bone biopsies were obtained on six of the author's first seven cases; in no instance was xanthomatosis found. Snapper makes a point of the fact that the biopsies were not taken from new lesions; indeed, he makes it appear that the very oldest lesions were purposely selected for the biopsies. Thus he states that their "Case 2 had been complaining about her right hip for 29 years . . . and that a biopsy was done on the oldest lesion in the crest of the ilium." The facts as published were that this patient, a housewife of 35, had been having pain in the right hip for 29 years; that the biopsy was taken from the crest of the ilium; that nothing was said about, and that there was no way of telling which was, the "oldest lesion in the crest of the ilium." As a matter of fact, one of the better arguments why Syndrome X is not a form of xanthomatosis is closely connected with the very good reason why biopsies were not taken from new lesions; it is one of the characteristics of this disease, in contrast to lipoid granulomatosis, that there seldom are new lesions. Of all the cases the author has seen, only his Case 3 showed any tendency of the bone lesions to progress. This important evidence from the bone biopsies can now be supplemented by a complete autopsy on our Case 3 (1) performed by Dr. H. Edward MacMahon (9); in spite of many sections of the bone lesions, this investigator could find only two nests of foam cells and felt that xanthomatosis was definitely ruled out. Sternberg and Joseph (10) came to the same conclusion from a complete autopsy performed on the case previously reported by McCune and Bruch (11).

As suggested in the first publication (1), the segmental distribution of the

bone and skin lesions is perhaps the strongest evidence against the etiology being a disorder of metabolism such as xanthomatosis. Thus, the author's Case 5 (1) had involvement of all the metacarpal and phalangeal bones of the left hand except for the three phalangeal bones of the index finger and the corresponding metacarpal bone, all four of which entirely escaped; none of the bones of the right hand was involved. It is not characteristic of a metabolic disease to stop in the midline or to run down one extremity and not the other; such is certainly not the case in instances of undoubted lipoid granulomatosis.

When one turns to the x-ray appearance of the bone lesions themselves, one finds many points of difference. In Syndrome X one finds not only bone destruction but areas where there is increased density of bone; in



FIG. 1. Eosinophilic granuloma of skull. Note circumscribed, punched-out areas (arrows) and complete absence of any increased bone formation (compare with Figure 2.) The details of this case were published by Thannhauser (28). (S. S., girl, age 4½.)

lipoid granulomatosis one finds only bone destruction. Whereas the author agrees with Snapper that lipoid granulomatosis need not involve the skull, in many cases of Syndrome X the skull is involved and, when it is, the lesions by x-ray are not at all like those in lipoid granulomatosis; instead of the sharply circumscribed punched-out holes, one finds, along with bone destruction, marked over-growth and thickening of the bone, an appearance not too unlike that seen in Paget's Disease (compare Fig. 1 with Fig. 2).

Another striking difference lies in the response to x-ray therapy: lipoid granulomatosis is very radio-sensitive: Syndrome X is radio-resistant.

The areas of cutaneous and buccal pigmentation which are such a striking feature of full-blown cases of Syndrome X are not characteristic of lipoid granulomatosis.

Finally, in Syndrome X, as in other conditions where the osteoblasts are hyperactive (osteitis fibrosa generalisata, osteitis deformans, etc.), the serum alkaline phosphatase level tends to be high; such is not the case in lipoid granulomatosis. As in osteitis deformans and probably in any condi-



FIG. 2. Skulls of three patients with Syndrome X. Note marked increased density at bases of skulls in A and B and characteristic overgrowth of bone in occipital region in C.

tion where the osteoblasts are hyperactive, one would anticipate that a certain percentage of cases with Syndrome X would develop sarcoma. The fact that the girl of 10 with "several small brown naevi" and precocious puberty reported by Snapper and Parisel (29) subsequently died of sarcoma is in the author's opinion some evidence in favor of an etiology other than lipoid granulomatosis.

### Is Syndrome X a Form of Neurofibromatosis of von Recklinghausen?

Since both Syndrome X and neurofibromatosis have bone lesions and areas of pigmentation, the question naturally arose, and still arises in the minds of some, whether they are not one and the same condition. Thannhauser (8), after a careful and scholarly study, has been unable to convince himself that they are separate entities; he even is not sure that the usual court of appeal, the pathologist, can distinguish them (*vide infra*).

If one grants for the moment that they are two separate entities, as the author firmly believes, in any case one would expect the literature of either one to contain misdiagnosed examples of the other. Thus the mere fact that a case in the literature diagnosed as one of the conditions had such and such findings is no proof that these findings are characteristic of the condition diagnosed. For example, before writing his first article the author combed the neurofibromatosis literature to find examples of Syndrome X; in this way was found, among the 35 cases of neurofibromatosis recorded by Stalmann (12), a typical example of Syndrome X (Stalmann's case 3), a female patient with precocious puberty and without cutaneous neurofibromata. One might cite this case, if one were not careful, as evidence that precocious puberty in females is a feature of neurofibromatosis.

To convince oneself that one is dealing with two conditions, all that is necessary is to select one feature for each condition which is common and pathognomonic of the condition and then see whether any cases can be found which exhibit the common-and-pathognomonic-features of both conditions. The author does not imply that any case which does not present the "common-and-pathognomonic-feature" is not suffering from the condition in question.

For the "common-and-pathognomonic-feature" of neurofibromatosis the author will select *multiple cutaneous neurofibromata*. Certainly most classical cases of neurofibromatosis contain such.

Before the feature selected for the common-and-pathognomonic one for Syndrome X is mentioned, it might be well to say a word about the bone lesions in neurofibromatosis. These in the author's opinion, based on his own experience and a review of the literature, consist of relatively few lesions. There are certain points of predilection, the commonest being the lower end of the femur and the upper end of the tibia (see Fig. 3). These





FIG. 3. Three cases of neurofibromatosis. These illustrate the marked tendency for the bone lesions to occur in the lower ends of the femora and the upper ends of the tibiae. In C note symmetrical lesions in upper ends of both tibiae and similar lesion in lower end of right femur; note in lateral view of right femur that lesion is from without in. B., kindly furnished by Dr. L. W. Gorham of Albany (C. W. L. 11 year old girl with neurofibromatosis previously reported by Gorham, Campbell, Howard and Donhauser (4)).

may represent points of entry for the nerves into the bone. The bone lesions themselves are exactly what one would expect if bone were encroached upon by a tumor of any kind, namely, sharply circumscribed areas of bone destruction with very little, if any, evidence of new bone formation. The cortex, to be sure, can be expanded by such a tumor just as it can be by a bone cyst. Most lesions can be demonstrated by suitable

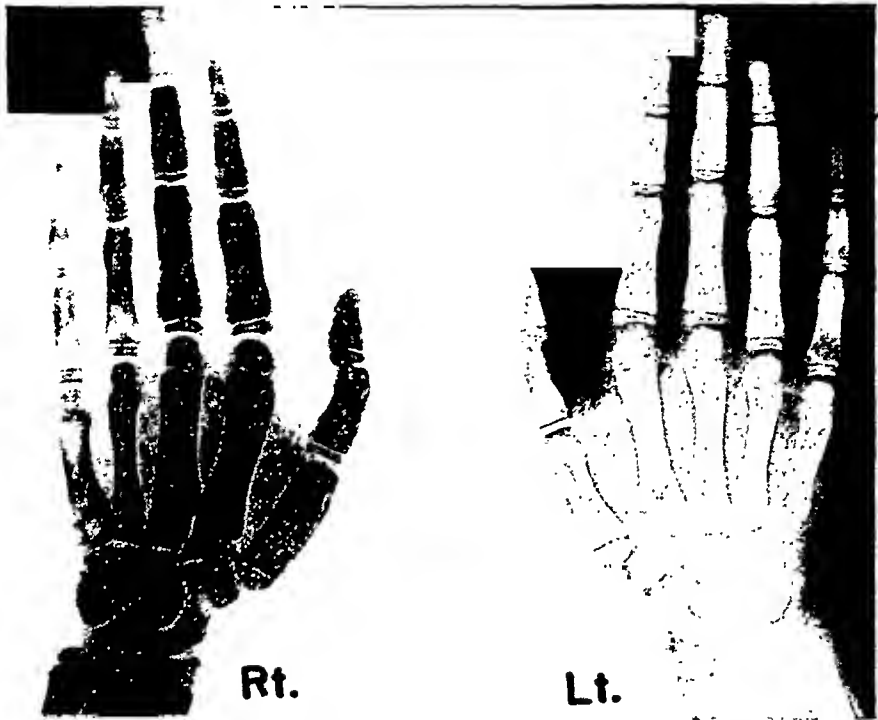


FIG. 4. Syndrome X. Note complete absence of lesions in left hand and extensive involvement of right hand; note fourth and fifth fingers of left hand escaped involvement; note that all the carpal bones of the right hand are involved, a somewhat unusual finding in this disease. (J.R., age 13.)

x-rays to arise from without, and to extend into the bone, rather than vice versa (see Fig. 3 C). The author is aware of no indisputable case of neurofibromatosis with very extensive bone involvement such as that of all the phalanges and metacarpal bones of a hand, or that of the entire skull including the base (see Figs. 2 and 4).

In contrast to the above, the bone lesions in Syndrome X in many instances are widespread; they usually show evidence of marked overgrowth of bone as well as bone destruction; and they commonly involve large areas of the skull including the base. Therefore, the author will select,

as the "common-and-pathognomonic-feature" of Syndrome X, *extensive bone lesions in which overgrowth of bone, as well as bone destruction, is a prominent feature.*

The author is aware of no case which presented the "common-and-pathognomonic-features" of both conditions. Certainly the eight cases which he and his colleagues reported (1, 2) and the many cases of Syndrome X which he has seen subsequently failed to show multiple cutaneous neurofibromata; certainly a not inconsiderable number of cases of neurofibromatosis which he has studied failed to show extensive bone lesions in which overgrowth of the bone was a prominent feature.

Thannhauser cites a series of cases (13 in all) with neurofibromatosis associated with osteitis fibrosa disseminata. An examination of this material shows that this association is very questionable.

Thus, the first 2 cases mentioned were published by Gould (13) (Gould's cases 1 and 4). They both had multiple cutaneous neurofibromata but the skeletal lesions were those of neither Syndrome X nor neurofibromatosis; both patients, as Gould pointed out and as the photomicrograph on Case 1 showed, had osteomalacia. The cause of the osteomalacia in the first case was probably renal acidosis as red granular kidneys were found at autopsy, and as a certain type of renal acidosis will cause osteomalacia (21); the cause of the osteomalacia in the second case was probably dietary as the patient died of extensive pulmonary tuberculosis.

Case 3 concerns a boy of 23 reported by Cohen and Douady (14) and previously reported by Leriche and Jung (15). The diagnosis of neurofibromatosis was beyond question; he had a multitude of cutaneous neurofibromata. The bone lesions were discovered accidentally when an x-ray was taken for an acute traumatic lesion of the right knee; this revealed a circumscribed "cyst" in the lower end of the right femur and another one at the upper end of the right tibia. The location and x-ray appearance of these cysts were absolutely characteristic of neurofibromatosis (*vide supra*); furthermore, an x-ray of his other knee revealed a similar "cyst" in the upper end of the left tibia. The case must be considered as neurofibromatosis with little to suggest Syndrome X.

Case 4 was a man of 36 years reported by Pagniez, Plichet and Fauvet (16). The evidence for Syndrome X was excellent: bone symptoms since the age of 3, widespread skeletal lesions confined to right side, areas of cutaneous pigmentation, and a high serum phosphatase level. However, there were only "deux petits mollusca" in addition to the cutaneous pigmentation to support the diagnosis of neurofibromatosis. The authors themselves thought that in the absence of fibromata the diagnosis of neurofibromatosis could not be retained.

Case 5, that of Mariante and Maciel (17), was finally dismissed by Thannhauser himself in a footnote wherein he stated that the bone lesions

would be better designated as "neurofibromatosis with bone cysts than osteitis fibrosa cystica disseminata."

Cases 6 through 10 are the five cases reported by Stalmann (12), that author's cases 2, 3, 4, 23 and 28. Case 6, (Stalmann's case 2), with a family history of neurofibromatosis and with cutaneous pigmentation and neurofibromata, undoubtedly had neurofibromatosis; from the data published it is impossible to diagnose the bone condition; there was a lesion of the right femur at the age of 2 which led to an inequality in the length of the legs and hence to marked scoliosis; there was also a lesion in the head of the right tibia; the localization of these lesions is most suggestive of neurofibromatosis (*vide supra*) but on the data available Syndrome X cannot be ruled out. Case 7 (Stalmann's case 3) was already mentioned above: a typical case of Syndrome X with nothing to suggest neurofibromatosis. Case 8 (Stalmann's case 4), a 35 year old female patient, had no cutaneous neurofibromata, but some small areas of pigmentation in the skin; she suffered from softening of the spinal column and of both femurs. The clinical diagnosis was osteomalacia and the x-rays of the pelvis showed typical findings of osteomalacia including "loosersche aufhellungszonen." There is little reason to believe that this case had either neurofibromatosis or osteitis fibrosa disseminata. Case 9 (Stalmann's case 23) had no cutaneous neurofibromata, in spite of a family history of neurofibromatosis; she menstruated at the age of 9; the bone lesions, which included involvement of the skull, strongly suggested Syndrome X. I am inclined to discount the family history and consider this case an example of Syndrome X without definite evidence of neurofibromatosis. Case 10 (Stalmann's case 28), a boy of 4, had no cutaneous neurofibromata: he, as well as his father, had specks of brownish pigmentation, probably freckles ("braune Flecke"); he was born with a deformity of the right tibia, the nature of which it is impossible to determine from the data given. I see little reason to believe that the patient had either neurofibromatosis or Syndrome X.

Case 11 (Case 1 reported by Uhlmann and Grossman (18)) was a classical case of neurofibromatosis with cutaneous neurofibromatosis and areas of pigmentation. There was only one bone lesion: this was in the right mandible; a histological section showed unmistakable neurofibroma. There was no reason to consider Syndrome X in differential diagnosis.

Case 12, a female of 47 reported by Ashton (19), probably did have neurofibromatosis with multiple cutaneous neurofibromata and "blotchy pigmentation . . . on the abdomen and chest, and in the form of freckles on the legs and arms." The bone manifestations of relatively short duration were probably Milkman's disease (20), a form of osteomalacia (21). She had symmetrical fractures of both femoral necks and the author described three types of rarefaction but failed to mention any overgrowth of bone.

Case 13, a female of 72 with acromegaly reported by Merklen and Israel

(22), like so many patients with this condition, did have many of the manifestations of neurofibromatosis (multiple cutaneous nodules and areas of pigmentation); however the description of the bone lesions is too inadequate for one to make a diagnosis. There was a marked kyphoscoliosis as one often sees in acromegaly, a slight involvement of the bones of the skull, a rarefaction of certain bones of the extremities, particularly of the right radius. There was a subcortical cyst in the latter bone with a fracture through it and a periosteal reaction in the region of the fracture. The author cannot rule out osteitis fibrosa disseminata from this evidence nor, however, can he rule it in.

So much for these 13 cases in which the two conditions were thought by Thannhauser to co-exist. In my opinion 6 had neurofibromatosis without evidence of Syndrome X, three of these six having osteomalacia as well; three cases had Syndrome X without evidence of neurofibromatosis; two cases had neither Syndrome X nor neurofibromatosis, one of the two having osteomalacia and the other probably osteogenesis imperfecta; the remaining two cases had neurofibromatosis combined with a bone disease in which the description was too inadequate to allow an accurate diagnosis.

There are several reasons to believe that Syndrome X is not a form of neurofibromatosis other than the fact that cases cannot be found containing the above mentioned "common-and-pathognomonic-features" of both conditions.

For one thing, neurofibromatosis has a strong hereditary tendency; so far Syndrome X, to my knowledge, has not been found to run in families.

A common feature of Syndrome X, but not of neurofibromatosis, is sexual-precocity-in-females. This interesting feature perhaps warrants a short digression.

In the author's opinion the onset of puberty is the result of the release of gonadotropic hormone or hormones from the anterior pituitary; this release in turn is due to stimuli coming over the hypothalamic-pituitary nervous pathway. By "true precocity" the author means the early release of gonadotropic hormone or hormones due to some disturbance in the hypothalamus. This is to be differentiated from precocity secondary to some functioning adenoma of a gland which produces a gonadal or gonadal-like hormone, e.g. granulosa cell tumor of the ovary producing estrin, an adrenal cortical tumor producing androgen, etc. According to this definition the precocity in Syndrome X is a true precocity. Thus case 2 in the author's series (1) had her first catamenia during her first year of life, and was still having periods at the age of 39; in spite of this she had been able to have children. To be sure, at the autopsy in the author's case 3 (1), Dr. H. Edward MacMahon found no evidence that ovulation had ever occurred, and Sternberg and Joseph (10) reported similar findings in

their case; therefore, if these two cases are to be considered as examples of true precocity one must exclude ovulation as a *sine qua non* for true precocity and apply the term to any precocity which is mediated through the hypothalamus. The prognostication made by the author and his colleagues that the cause of the precocity is some lesion in the region of the hypothalamus received support from the above-mentioned autopsy by Dr. H. Edward MacMahon. This patient, who had had her first menstrual period before the age of 1, showed a marked diminution in size of one mammillary body and an accessory nucleus in the adjacent tissue. On the other hand, Sternberg and Joseph (10) failed to find any lesion in the hypothalamus in their case but did find marked hyperplasia of the basophile cells of the pituitary, which suggests that these cells were being stimulated by some influence, possibly a disturbance originating in the hypothalamus.

There is an alternative explanation suggested by Thannhauser which cannot be dismissed. He would explain the precocity on the basis of pressure on the hypothalamus secondary to the overgrowth of bone at the base of the skull. It seems just possible that the changes in the mammillary body found at autopsy in our case 3 (*vide supra*) might be the result of long-standing pressure on this region. With the exception of our case 2, who had marked precocity but no definite thickening at the base of the skull by x-ray, it does seem that most of the cases of precocity did have considerable thickening at the base of the skull.

It is interesting that no case of Syndrome X with marked sexual precocity has been described in the male. Furthermore, tumors in the region of the pineal body cause precocity only in males,—the so-called "pineal syndrome." Apparently the mechanism which releases puberty in the male is different from that which releases it in the female. The author and his colleagues in their first publication (1) attempted to explain this discrepancy between males and females on the ground that the luteinizing hormone of the anterior pituitary leads to androgen formation whereas the follicle-stimulating-hormone leads to estrin formation. It seemed possible that, because of some hypothalamic disturbance in Syndrome X, there was a precocious production of follicle-stimulating-hormone but not of luteinizing hormone; perhaps in keeping with this hypothesis are the autopsy findings cited above of complete absence of any evidence of recent or old corpora lutea, since these bodies require for their production luteinizing hormone.

Thannhauser (8) makes an important point of the fact that he found four cases of neurofibromatosis combined with precocious puberty. However, three of these cases occurred in males and so can not be considered as evidence for a similarity between neurofibromatosis and Syndrome X. The fourth case, reported by deVries (23), was a girl with somatic and sexual

precocity who had her first period at the age of 9 and who clinically showed evidence of a pituitary tumor. There were no bone lesions. The evidence for neurofibromatosis rested on a few areas of brownish pigmentation and one neurofibroma of the skin of the foot.

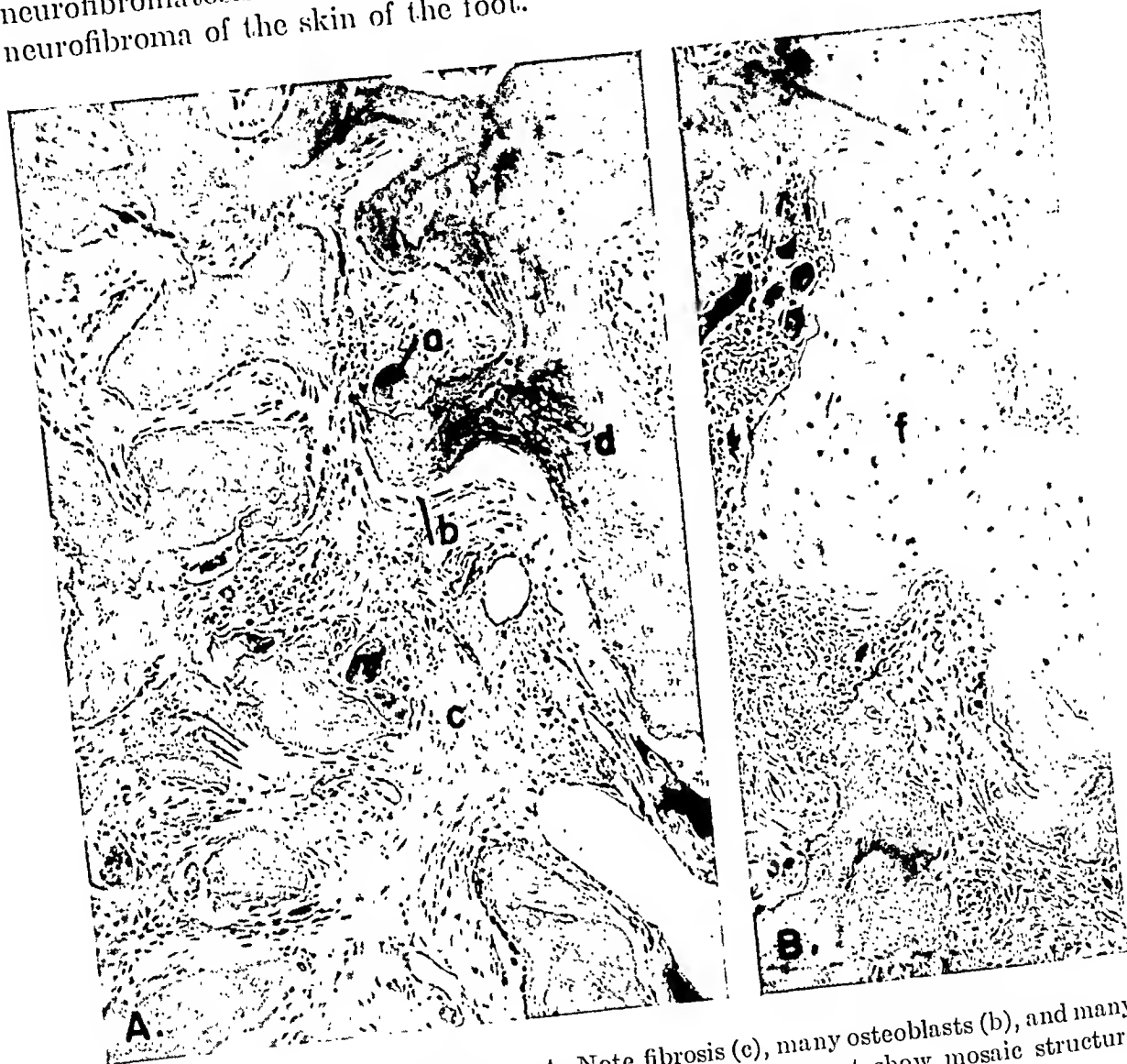


FIG 5. Syndrome X: bone biopsy. A. Note fibrosis (c), many osteoblasts (b), and many osteoclasts (a); note furthermore, that trabeculae (d) do not show mosaic structure characteristic of Paget's disease. B. Note island of cartilage (f).

Thannhauser believes that the histology of the bone lesions of neurofibromatosis and of Syndrome X may be indistinguishable. He points out that both may show dense cellular tissue, with or without whorls. The author grants that they may be indistinguishable if in a case of Syndrome X one selects an area in the center away from bone and compares it with a section from a neurofibroma. However, in Syndrome X one seldom finds a sizeable biopsy which does not contain some evidence of bone formation;

in neurofibromatosis of bone on the other hand one frequently does. If one takes a section through an area where bone is still present, there can be no question as to the diagnosis (compare Fig. 5 with Fig. 6). Furthermore, as pointed out by the author and his colleagues in their first publication (1), in Syndrome X one not infrequently finds areas of cartilage some of which



FIG. 6. Neurofibromatosis: bone biopsy. Note bone trabeculae (a), foam cells (b), and whorls (c). Foam cells are found in neurofibromatosis and probably represent degenerative changes. Photomicrograph was kindly furnished by Dr. Benjamin Castleman and pertains to the same lesion as x-ray shown in Figure 3 (A).

almost certainly are derived from the epiphyseal cartilage (see Fig. 5 and Fig. 7).

What about the areas of pigmentation? The author believes that in nine cases out of ten even these are different in the two conditions. In Syndrome X the areas have an irregular outline like the coast of Maine, whereas in neurofibromatosis the outline is more reminiscent of the coast of California (see Fig. 8). To be sure the author has seen one case of undoubted neurofibromatosis with areas of pigmentation very similar to those seen in Syndrome X.

A final point of difference is the not infrequent occurrence of elephantiasis in neurofibromatosis, whereas this condition has not yet been reported in Syndrome X.



## WANTED: A NAME

The author, having satisfied himself and, he hopes, the reader, that the syndrome under discussion is an entity, again opens the question as to what to call it. A satisfactory answer to this question has not been found in the past, nor is one forthcoming now. Since the etiology of the syndrome remains quite obscure, the author and his colleagues, Drs. Allan M. Butler,

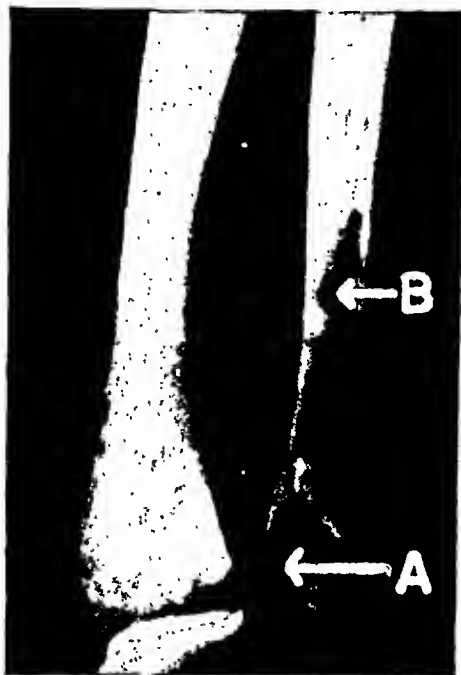


FIG. 7. Syndrome X. X-ray of the forearm to show that some bone lesions may be the result of a dyschondroplasia. Note marked irregularity of epiphyseal cartilage at A which strongly suggests that islands of cartilage are being incorporated in the diaphysis. B. represents such an island.

Aubrey O. Hampton and Patricia Smith in their first publication used the descriptive designation, "Osteitis Fibrosa Disseminata with Areas of Cutaneous Pigmentation and an Endocrine Dysfunction with Precocious Puberty in Females." Because of the unwieldiness of this terminology and because A precedes B, H. and S. in the alphabet and probably for no other reason the term, Albright's Syndrome, came into being.

The use of a person's name for the designation of a syndrome has objections, the chief one being that no one can decide whose name to use. One can always go to the literature and find some preceding reference to a case which in all likelihood had the syndrome in question. Often each language produces its own "first" describer. In this connection the present author, a number of years ago, ran into a rather amusing state of affairs. The question as to the terminology of Syndrome X had come up and he thought it would be of interest to look over vonRecklinghausen's original

1891 monograph, which includes the description of a number of different bone conditions, to see whether by any chance a case with Syndrome X had been described. It turned out that one and probably two of the 3 famous cases in the monograph (cases 5, 6 and 7), which had been considered as examples of osteitis fibrosa generalisata and hence of hyperparathyroidism, most surely had not been afflicted with hyperparathyroidism but with Syndrome X. Fortunately, as the present author pointed out at that time (24), the third case (case 7) in all probability did have hyper-



FIG. 8. Pigmentation in neurofibromatosis compared to that in Syndrome X. Note "coast-of-Maine" contour of areas of pigmentation in A as opposed to "coast-of-California" contours in B. Incidentally, note subcutaneous nodules in B and their absence in A. (A.—A.K. with Syndrome X; B.—E.G. with neurofibromatosis.)

parathyroidism; otherwise we would be confronted with the disturbing state of affairs that von Recklinghausen had neglected to describe what is now known as von Recklinghausen's disease of bone. It will be seen, therefore, that as far as the bone manifestations of the syndrome are concerned von Recklinghausen preceded the author and his colleagues by 46 years. Thus there is as much reason to connect von Recklinghausen's name with this bone disease as with osteitis fibrosa generalisata; indeed, since cases 5 and 6 with osteitis fibrosa disseminata preceded case 7 with osteitis fibrosa generalisata, Thannhauser makes the utterly logical, if somewhat impractical, suggestion that Syndrome X be designated, "osteitis fibrosa disseminata (von Recklinghausen)," and that the name of von Recklinghausen be dropped from osteitis fibrosa generalisata. In other words, he would have us stop using von Recklinghausen's name for the bone disease which we have learned to associate with it and apply it to another disease: furthermore he would put into the mouth of von Recklinghausen, dead these many years, the term, osteitis fibrosa disseminata, coined by the

author and his colleagues. In the end, for those who believe with Thannhauser that Syndrome X and neurofibromatosis are one and the same thing, this would lead to a simplification of terminology since there would be only one "von Recklinghausen's disease."

There is probably one simple way out of the above dilemma. After all, the most striking feature of the syndrome is perhaps the bone disease. A descriptive name for the bone disease could be found and the whole disease could go under this name. This is exactly what is taking place. The tendency is to use the name, "polyostotic fibrous dysplasia,"\* suggested by Lichtenstein (25), rather than the name, "osteitis fibrosa disseminata," suggested by the author and his colleagues. The present author has no fault to find with this tendency. A case with precocity and cutaneous pigmentation but without evidence of bone lesions such as that described by Kurzrok (27) could be designated "polyostotic fibrous dysplasia sine fibrous dysplasia."

#### SUMMARY

1. This paper concerns the syndrome which in its complete form is characterized by 1) a disseminated osteitis fibrosa (both hyper-and-hypo-ostotic) with a segmental distribution, 2) areas of cutaneous pigmentation which have a distribution suggesting some connection between them and the bone lesions, and 3) sexual and somatic precocity when the condition occurs in the female.

2. The author believes this syndrome is not a form of lipoid granulomatosis (xanthomatosis) because:

- a) the blood cholesterol level is not abnormal (a minor piece of evidence);
- b) bone biopsies show "foam cells" only infrequently;
- c) the bone lesions show only a slight tendency to progress, clear up spontaneously, and are not radio-sensitive;
- d) the segmental distribution of the bone and skin lesions is not suggestive of a metabolic disorder;
- e) the x-rays show increased bone formation as well as bone destruction;
- f) the areas of cutaneous pigmentation are not characteristic of lipoid granulomatosis;
- g) when the disease is widespread the serum phosphatase level is high; and
- h) sexual-precocity-in-females is not a feature of lipoid granulomatosis.

3. The author believes this syndrome is not a form of neurofibromatosis (von Recklinghausen) because:

- a) he has never seen a case nor found one in the literature where multiple cutaneous fibromata, a common-and-pathognomonic-feature of neurofibromatosis, and widespread bone disease with evidence of increased as well as decreased bone forma-

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\* Lichtenstein and Jaffe (26) subsequently omitted "polyostotic" in order to include those cases where the condition is confined to one bony lesion and simply called the disease "fibrous dysplasia."

- tion, a common-and-pathognomonic-feature of the syndrome under discussion, were present in the same individual;
- b) the syndrome does not tend to run in families;
  - c) sexual-precocity-in-females is not characteristic of neurofibromatosis although sexual precocity, especially in males, occasionally occurs because of a neurofibroma in the region of the hypothalamus (pineal syndrome);
  - d) an autopsy by Dr. H. Edward MacMahon on a patient with the syndrome showed as a possible cause of the precocity not a tumor but a lesion in one mammillary body;
  - e) the bone lesions in neurofibromatosis are not extensive, do not show new bone formation, and are confined to certain localities, notably the upper ends of the tibias and the lower ends of the femurs;
  - f) the areas of cutaneous pigmentation in neurofibromatosis usually have smooth edges like the coast of California rather than the irregular edges like the coast of Maine which characterize the areas in the syndrome under discussion;
  - g) elephantiasis, so common in neurofibromatosis, has not been found in this syndrome.

4. The terminology is discussed with the conclusion that the condition had best be termed "polyostotic fibrous dysplasia" as suggested by Lichtenstein.

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# THE ACTIVITY OF ARGINASE IN RED BLOOD CELLS

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KOCHAKIAN (5) found that the red blood cell arginase activity was increased in one patient with the adrenogenital syndrome, and lowered in two with Addison's disease. Fraenkel-Conrat, Simpson and Evans (2) reported an increase in the activity of liver arginase in hypophysectomized rats following the administration of 17-hydroxydehydrocorticosterone, dehydrocorticosterone, and corticosterone. The present paper attempts to obtain additional evidence on these points.

## METHODS

1. **Preparation of laked red blood cells for arginase determination:** Blood was taken in heparin, 15 ml. for preparation of the standard reference curve, and 10 ml. for subsequent determinations. After removal of 3 ml. for the hemoglobin determination, the remainder was centrifuged in 6 ml. tubes to obtain the hematocrit. The plasma was discarded and the red cells washed once with isotonic saline. The packed cells were then diluted to five times their volume with distilled water and shaken occasionally for 10 minutes to insure complete hemolysis. Aliquots of this solution of hemolyzed cells were used in the arginase determinations.

2. **Preparation of the standard reference curve:** The following method, used for the preparation of a standard reference curve of arginase activity from a solution of hemolyzed red cells, is a combination of methods used by Hunter and Dauphinee (3) and Hunter and Downs (4) and influenced by those of Edlbacher and Rothler (1) and Kochakian (6).

Four 10 ml. volumetric flasks were set up, each containing 2 ml. of 1.875 per cent arginine-hydrochloride solution at pH 7.0. Four additional flasks were set up as controls, containing 2 ml. water. Into each of the 8 flasks 2 ml. of 0.5 M disodium phosphate solution, pH 8.8, were measured. Finally, into these arginine and control flasks were placed successively the aliquots 0.5, 1.0, 2.0, and 3.0 ml. of the solution of laked cells. Water was added to bring the volume in each flask to 8 ml. After being thoroughly shaken, the group of 8 flasks was incubated at 37° for 6 hours, at the end of which time arginase activity was stopped by placing the flasks in boiling water for 5 minutes (3). At this point, a drop of caprylic alcohol was

usually added to each flask, and the flasks were made up to within 3 drops of 10 ml., transferred to test tubes, and centrifuged. The supernatant, poured off the precipitated protein, was neutralized to approximately pH 7.0 by the addition of 3 drops of 0.5 N HCl. A 2 ml. aliquot of each solution was analyzed for urea by the Van Slyke manometric method (9) for urea in whole blood or serum. Each urea determination of the four original arginine flasks was corrected by a urea done on its corresponding control.

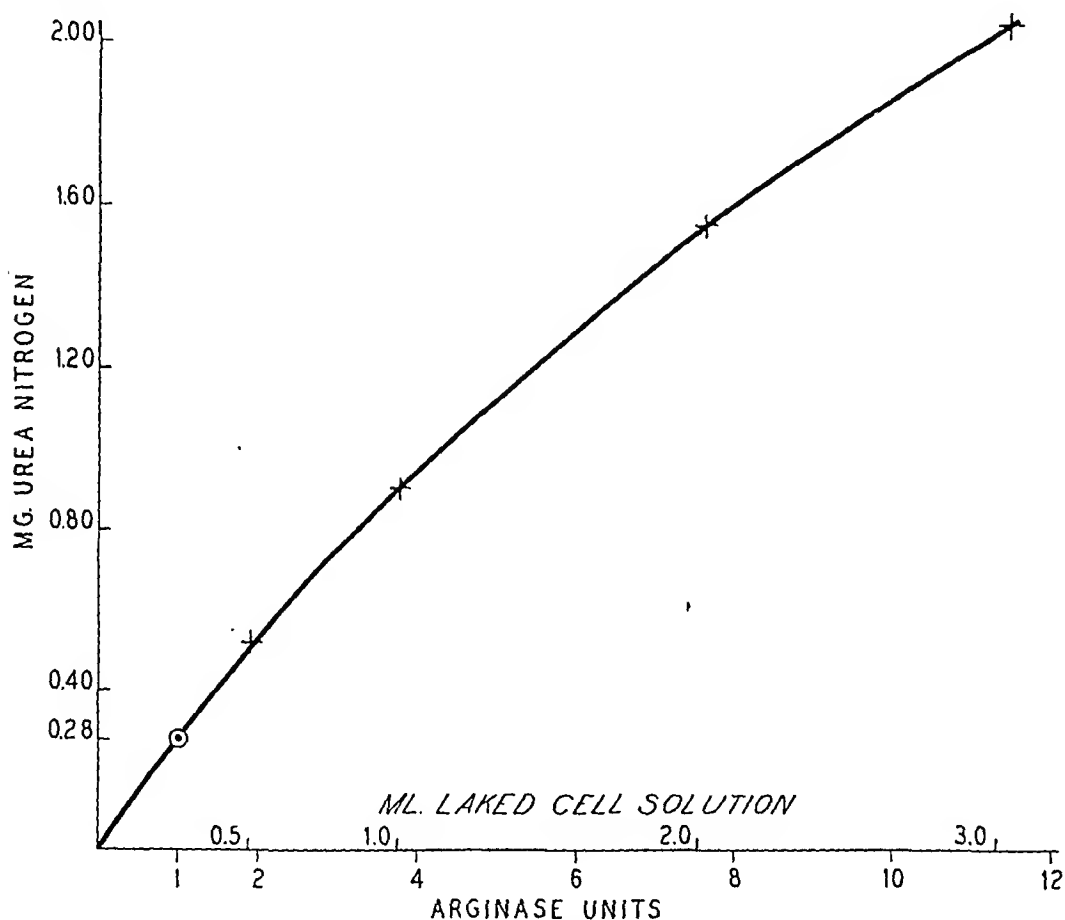


FIG. 1. Standard reference curve for measurement of arginase activity in red blood cells.

A curve (Fig. 1) was constructed by plotting milliliters of laked cells as the abscissa against milligrams of urea nitrogen formed as the ordinate. On this curve an arbitrary point was chosen to define the unit of arginase activity. This point represented the amount of arginase in the laked cell solution necessary to produce 0.28 mg. of urea nitrogen under the conditions of the experiment. The abscissa was then laid off in these units. The unit of arginase thus defined represents 1/50 milliequivalent of urea or ammonia nitrogen, and is equivalent to the arginase unit defined in other methods as the amount of ammonia titrated by 1 cc. N/50 HCl. In order to have an

appreciable excess of the substrate present during the reaction, the curve was determined for only 2 mg. of the available 5 mg. of urea nitrogen, and to increase accuracy further, it was used only between the points represented by 0.5 and 1.8 mg. of urea nitrogen.

3. **Determination of arginase in red blood cells:** The procedure for the determination of arginase in unknown bloods was the same as that described for the preparation of the curve, except that only two aliquots, 2.0 and 3.0 ml., and a pair of controls were used. From the value in arginase units obtained from the curve, the final value, calculated for each duplicate urea determination, was expressed in arginase units per ml. of packed red blood cells. A difference between duplicate determinations of 1.0 A.U. per ml. RBC was tolerated in a few cases, although the difference was usually lower, averaging 0.4 A.U. per ml. RBC.

4. **Provision of a second reference standard for arginase:** Arginase activity, in addition to being referred to packed cells, was also referred to hemoglobin, which was determined by the Van Slyke-Neill oxygen capacity method (8, 10).

### FINDINGS

The arginase units are reported in milliliters of packed red cells. The values referred to hemoglobin, although higher, paralleled these too closely to need to be reported separately.

Nine normal women (Table 1) varied from 8.4 to 16.3, average 11.2, while seven normal men ranged from 5.4 to 11.3, averaging 8.9. These findings do not support the suggestion that androgens increase the arginase activity.

The physiological variation (Table 2) was determined in three indi-

TABLE 1. NORMAL MEN AND WOMEN, AGE GROUP 20-40  
Arginase units per ml. RBC

Women	Men
8.4	5.4
8.5	6.0
9.6	6.2
9.6	6.4
9.9	7.1
11.6	9.7
11.8	11.3
15.0	
16.3	
Average 11.2	Average 8.9



viduals by making for each three observations over a period of a month, and was about one unit. In one case a determination six months later was similarly constant. No change was observed at different times in the menstrual cycle.

TABLE 2. PHYSIOLOGICAL VARIATION FROM DAY TO DAY  
Arginase units per ml. RBC

	6 mos. later			
G.C., normal woman	9.7	9.9	10.2	9.6
G.S., convalescent coronary man	15.2	15.4	13.8	
M.G., hyperthyroid man	6.5	6.9	6.3	

Methyl testosterone, 50 mg. daily, was given to two normal males for six days, and the changes were about one unit (Table 3).

One patient with Simmonds's disease and one with Addison's disease received 11-dehydrocorticosterone (Table 4). The former showed a marked increase in red cell arginase from 5.7 to 16.0 units, the latter remained constant at about 15.0 units. Metabolic studies on these patients are reported by Perera, Blood and Reinhold (7).

TABLE 3. EFFECT OF METHYL TESTOSTERONE ON TWO MALE PATIENTS  
Arginase units per ml. RBC

Patient	Before M.T.	After receiving 50 mg. M.T. for 6 days
A.G.	5.5	5.7
R.B.	11.4	9.3

TABLE 4. EFFECT OF 11-DEHYDROCORTICOSTERONE ACETATE  
Arginase units per ml. RBC

Disease	Before DHCA	After receiving DHCA for 7 days	
Simmonds's	5.7	16.0	20 to 40 mg. daily
Addison's	15.6	14.5	100 mg. daily

Fifteen units of antianemia liver extract were given to five non-anemic convalescents for five days; there was no change in the red cell arginase for the next fifteen days.

Determinations were carried out in various diseases (Table 5). Two cases of Addison's disease, taking doca, have rather high values as opposed to the low values reported by Kochakian. Aside from these cases there seems

to be no definite order, and it appears that the red cell arginase concentration varies with each individual rather than with the disease, or with any of the observed endocrine disorders, except Simmonds's disease.

TABLE 5. PATIENTS

A.U. per ml. RBC	Sex	Diagnosis	Age
3.9	M	Infectious hepatitis	14
4.2	M	Pneumococcus pneumonia	42
4.3	M	Infectious hepatitis	59
5.1	M	Peptic ulcer (bleeding)	42
5.7	M	Simmonds' disease	36
6.6	M	Hyperthyroidism	47
7.5	M	Peptic ulcer (bleeding)	51
7.5	M	Duodenal ulcer (bleeding)	35
8.3	M	Duodenal ulcer (bleeding)	78
8.9	F	Hyperthyroidism	41
9.7	F	Atypical Cushing's syndrome (17 ketosteroids, 17.4 mg. per 24 hrs.)	16
9.9	M	Pneumococcus pneumonia	38
10.0	F	Rheumatic fever	39
10.1	F	Infectious hepatitis	31
10.2	M	Hodgkins' disease	38
10.3	F	Anorexia nervosa	56
10.4	F	Essential hypertension	53
10.6	F	Infectious mononucleosis	23
11.4	M	Coronary occlusion	70
11.9	M	Ulcerative colitis	15
12.8	F	Hyperthyroidism	35
13.5	M	Coronary occlusion	50
13.5	M	Coronary occlusion	62
13.8	M	Coronary occlusion	40
14.9	M	Coronary occlusion	53
15.5	M	Rheumatic heart disease	31
15.6	M	Addison's disease	31
16.2	M	Duodenal ulcer (bleeding)	84
16.7	F	Infectious mononucleosis	23
17.6	F	Cirrhosis	30
18.3	F	Addison's disease	30
19.7	F	Gaucher's disease	32
22.0	F	Infectious mononucleosis	30
29.0	M	Chronic pancreatitis (?)	50

## SUMMARY

The red blood cell arginase activity is quite constant in a given individual. It is slightly higher in women than in men and is not influenced in

normal men by methyl testosterone. The range does not appear to vary in the diseases studied, with the possible exception of higher values in infectious mononucleosis. A marked rise in one case of Simmonds's disease was produced by 11-DHCA.

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# THE EXCRETION OF 11-OXYCORTICOSTEROID-LIKE SUBSTANCES BY NORMAL AND ABNORMAL SUBJECTS\*

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## INTRODUCTION

THE present paper reports approximately 300 measurements of the urinary output of 11-oxycorticosteroid-like substances (11-OCS) by a group of normal subjects and by selected patients with various conditions. The purpose of these studies has been to clarify the significance of the urinary content of these substances with particular reference to variations in adrenal cortical function in health and disease.

It is the 11-oxycorticosteroids which have been found to promote sugar formation from protein (i.e., protein catabolism) and to play a role in the resistance of the organism to the stresses and strains of various traumatic experiences (1, 6). Because of their action on sugar metabolism, they are called the "S-hormones." They are to be distinguished from other cortico-adrenal hormones certain of which are androgenic and prompt protein anabolism (N hormone) and others of which act chiefly to cause retention of water, sodium and chloride and excretion of potassium ( $H_2O$  and electrolyte hormone).

## METHOD

The 11-OCS output was measured by means of a colorimetric assay procedure reported elsewhere (8). The substances measured are thought to correspond to corticoadrenal steroids which have a ketone or hydroxyl group on the 11th carbon atom and which have attached to the 17th carbon atom a 2-carbon, sugar-like or ketolic side chain and an hydroxyl group.

In evaluating the results reported here, it is important to bear in mind the approximate sensitivity of the method. The smallest quantity of crystalline 11-oxycorticosteroid which can be measured accurately in the final colorimetric assay is about 0.018 mg. Smaller amounts can be assayed only approximately. Because it is inherent in the analytic procedure that only a

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fraction (usually about  $1/6$ ) of the urine sample extracted can be submitted to the final colorimetric analysis, the least amount in a urine specimen which can be measured accurately is approximately 0.11 mg. In the majority of normal urines reported here, however, only about  $3/5$  of the 24-hour urine sample was extracted. Hence the lowest accurate value in this series was about 0.17 mg. per 24 hours. In this connection it will be seen that the average values obtained in the majority of normal subjects were 0.17 mg. or more per day. In patients suspected of hypoadrenocorticism the tendency was to extract the entire 24-hour sample. The urines of patients with abnormally high values were treated as for the normal subjects.

### RESULTS

*Normal Subjects.* Eighty-three 24-hour excretion values were obtained on 20 normal young adults, 12 of whom were men and 8 of whom were women. The average for the entire group was 0.22 mg. per day. One hundred per cent of the values obtained fell between 0.10 and 0.44, 90 per cent between 0.12 and 0.37, 80 per cent between 0.12 and 0.32 and 70 per cent fell between 0.13 and 0.32 mg. per day, respectively. No significant difference between the values for the sexes could be made out.

While the foregoing suggests that the lower limit of normal 11-OCS output was 0.10 to 0.12 mg. per day, further analysis of the data disclosed that it should perhaps be placed higher. When averages\* of two or more consecutive 24-hour assays were arranged in order of decreasing magnitude, it was noted that 44 per cent of them were 0.20 mg. or less, 31 per cent were 0.19 mg. or less and only 6 per cent were 0.18 mg. or less. Thus an average value for two or more consecutive determinations of less than 0.18 mg. per 24-hour sample would be lower than the average values of 94 per cent of normal subjects studied to date.

The chances of obtaining an unusually low or high value for an individual in a single assay are illustrated in Figure 1. Here are shown the day by day output values for 3 normal men and 1 normal woman. For these data the chances were 2 out of 3 that the value for a single day would fall within plus or minus 30 per cent of the average value for that individual. The figure indicates, however, that especially when there is a question of abnormally low 11-OCS excretion, a series of determinations would give much more accurate information than a single measurement.

To investigate the possibility of using 8 or 12-hour urine collections instead of 24-hour collections the experiment recorded in Figure 2 was performed. Here 7 normal young men collected their urine over 8-hour periods for  $2\frac{1}{2}$  days. On the first 2 days they lived a relatively sedentary existence;

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\* Sixteen such average values were available.

on the morning of the third day they took a brisk 5 mile walk. While all the subjects were in good health, only about half were considered to be in good physical trim. The figure shows a slight tendency for the 11-OCS output to be highest in the forenoon and lowest at night. The 5-mile walk was without significant influence upon the 11-OCS output. The 24-hour values found by obtaining the sum of the 3 respective 8-hour values for

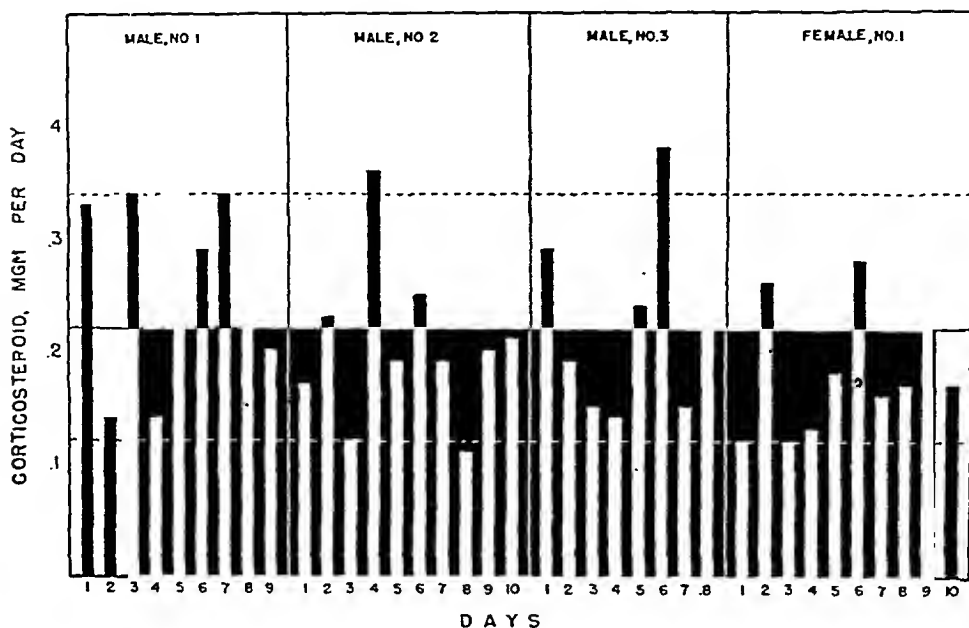


FIG. 1. Day by day output of 11-OCS by 3 normal young men and 1 normal young woman. The continuous horizontal line intersecting the ordinate at about 0.22 mg. represents the average value for this series; the interrupted horizontal lines give the range for 90 per cent of the values.

each day (right hand section of Figure 2) corresponded quite closely to ordinary 24-hour assays obtained on these and other normal individuals. However, the prediction of 24-hour values from individual 8-hour assay figures yielded irregular and insignificant results.

*Hypoadrenocorticism.* Section A of Table I records 25 assays on 17 patients with this condition. The average 11-OCS output for the group was 0.14 mg. per 24 hours (range 0.02 to 0.29 mg.). About two-thirds of these values were 0.18 mg. or less and 59 per cent were 0.17 mg. per day or less. While there appeared to be a definite tendency toward abnormally low values in these patients, a number of them had one or more assays which were well within normal limits. Attempts to discover a clear cut relation

between the 11-OCS output and the presence of such a metabolic disturbance as hypoglycemia were unsuccessful.

Though many of the patients were receiving desoxycorticosterone acetate therapy at the time of the urine collection, no consistent relation between this therapy and the 11-OCS excretion could be determined.

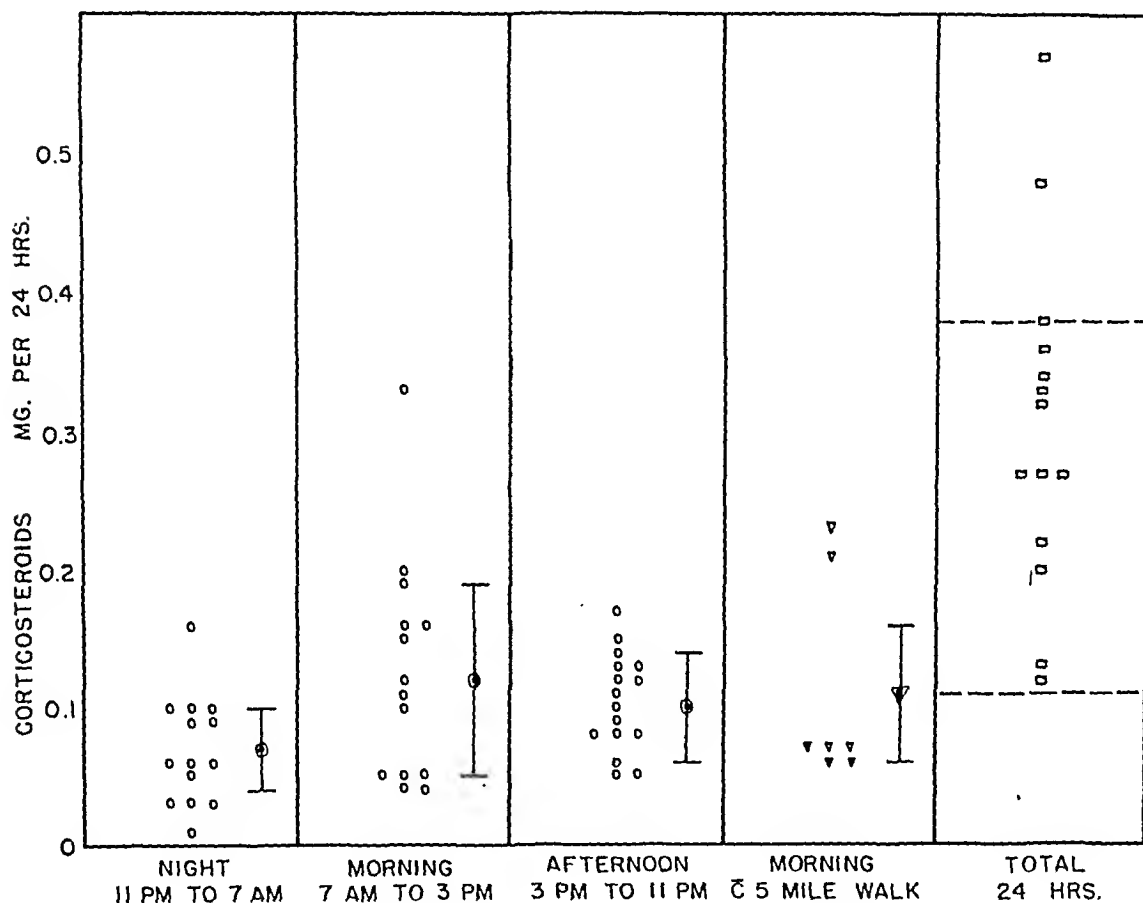


FIG. 2. Variation in 11-OCS output at different times of day; effect of 5 mile walk upon 11-OCS output. With the exception of the right hand section the values are for 8 hour collection periods over the designated times. The right hand section gives the 24 hour values obtained by adding the three 8 hour values of each subject each control day. The interrupted horizontal lines in this section correspond to those of Figure 1.

Thus it appears that the administration of this agent has no measurable effect upon the 11-OCS output as determined here.

*Hypothyroidism.* Section B, Table I, presents data on 2 women with classical myxedema. In both the 11-OCS values were low. The effect of thyroid therapy on the 11-OCS output of one of these patients is illustrated in Figure 3. Whereas the 11-OCS output was abnormally low prior to therapy, it rose to normal levels promptly after thyroid medication was

instituted. It is of interest that relatively large doses of thyroid (300 mg. per day) did not result in an abnormal elevation or depression of the 11-OCS output.

*Hypopituitarism.* Section C of Table I sets forth observations on 7 patients with this condition. There was a distinct tendency towards abnormally low values. Four were less than 0.10 mg. and 6 of the 7 were less than 0.18 per day. It may be significant that the lowest values were

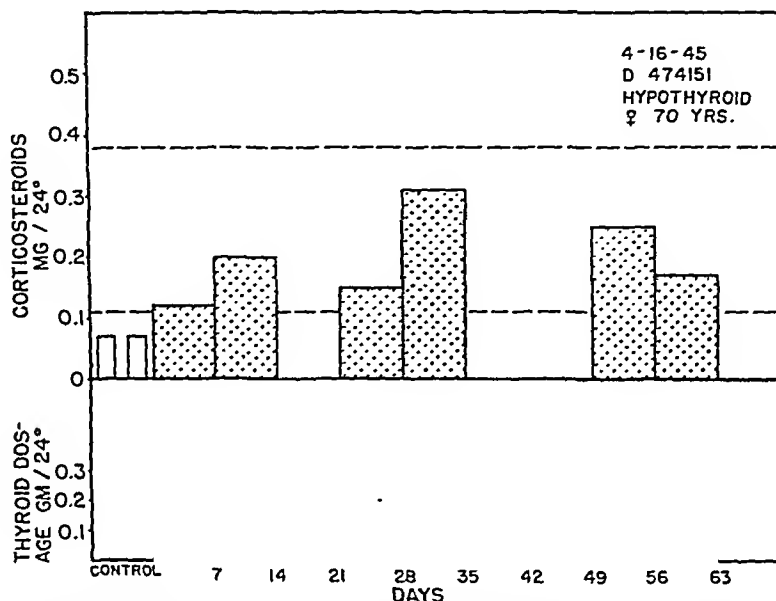


FIG. 3. Excretion of 11-OCS by an hypothyroid patient (No. 18, Table I) before and after U.S.P. thyroid therapy. The interrupted horizontal lines correspond to those of Figure 1.

obtained on the patients who showed the most distinct symptoms of hypo-adrenocorticism.

*Hyperadrenocorticism with Virilism.* Section D, Table I, gives information on 6 patients with this disease. It will be noted that all but one of these had proven or presumed adrenal cortical hyperplasia as the underlying pathologic process; in the one exception there was an obvious and very extensive neoplasm of the adrenal cortex. It will also be seen that symptoms and signs characteristic of Cushing's syndrome were lacking. Without exception the urinary 17-ketosteroid values were abnormally elevated. However, except for the patient with adrenal neoplasm (No. 32) the 11-OCS values were either within normal limits or only slightly elevated: the value in patient No. 32 was definitely abnormally high (1.45 mg. per day). On the other hand, while the 17-KS value in this patient was



TABLE I. EXCRETION OF URINARY CORTICOSTEROID-LIKE SUBSTANCES (11-OCS)  
AND OF 17-KETOSTEROIDS (17-KS) BY PATIENTS WITH VARIOUS CON-  
DITIONS. THE VALUES GIVEN ARE MILLIGRAMS PER 24 HOURS

Patient Number	Description	17-KS	Urinary 11-OCS		
			No. of Determinations	Av.	Range
<i>A. Hypoadrenocorticism</i>					
1	Male, age 45, with history of renal TBc, and with microcardia, hypotension and generalized pigmentation.	3.2	1	.03	—
2	Male, age 18, moderately severe idiopathic hypoadrenocorticism of 6 yrs. duration. Moderate marked weakness. No spontaneous hypoglycemia.	3.6	1	.06	—
3	Male, age 38, with TBc, moderate marked muscle weakness and tendency to hypoglycemic episodes.	1.9	1	.07	—
4	Female, age 49, with Addison's disease for 1 yr. Very weak but no clear evidence of hypoglycemia.	0.8	2	.11	.06-.16
5	Male, age 51, with Addison's disease of 5 yrs. duration. Active pulmonary TBc. Calcification of adrenals by x-ray.	4.4	1	.11	—
6	Male, age 45, with moderately severe Addison's and quiescent TBc. Moderate muscle weakness and irregular tendency to hypoglycemia.	2.5	3	.11	.06-.16
7	Male, age 41, with recently diagnosed hypoadrenocorticism and with recent slight gynecomastia. No hypoglycemia. Moderate muscle weakness.	4.2	4	.14	.08-.24
8	Male, age 29, with Addison's disease of 2 yrs. duration. One undescended testicle and BMR of -27%.	3.2	2	.14	.09-.18
9	Female, age 45, with striking tendency to hypoglycemia which was prevented by 3 cc. adrenal extract daily. Also tendency to edema when receiving more than 2 mg. DOCA daily. BMR -18% on 60 mg. thyroid.	—	1	.16	—
10	Male, 35 years, with weakness, pigmentation. No hypoglycemia except after prolonged fast. Normal glucose tolerance. See also Figure 8.	3.3	1	.17	—
11	Female, age 52, with mild Addison's disease, slight pigmentation; essentially normal insulin tolerance.	1.0	1	.18	—
12	Male, age 49, with Addison's disease for 13 yrs. Maintained well with DOCA pellets. Crises only when DOCA discontinued. No striking tendency to hypoglycemia.	2.8	1	.19	—
13	Female, age 32, with Addison's disease of 6 yrs. duration. Onset following severe hemorrhage at end of pregnancy. Develops edema very easily on small doses of DOCA. BMR -35%. Many severe hypoglycemic episodes.	2.6	1	.20	—
14	Male, age 38, without gross tendency to hypoglycemia.	4.4	1	.21	—
15	Male, age 27, with Addison's disease of about 10 years duration. Tuberculous kidney removed 6 yrs. ago. Has crises when DOCA therapy stopped and rather marked tendency to spontaneous episodes of hypoglycemia. BMR -15%.	2.0	2	.24	.18-.29

TABLE I—(continued)

Patient Number	Description	17-KS	Urinary 11-OCS		
			No. of Determinations	Av.	Range
16	Male, age 43, with Addison's disease of 6 yrs. duration. Doing well on DOCA pellets. No striking tendency to hypoglycemia. Weight loss and weakness develop rapidly if therapy is withdrawn. BMR -21%.	4.4	1	.23	—
17	Female, age 20, with severe Addison's disease of 4 yrs. duration and with tendency for spontaneous hypoglycemia. BMR -18%.	0.2	1	.23	—
	SEE ALSO PATIENT NO. 31. <i>B. Hypothyroidism</i>				
18	Female, age 69, with classical myxedema following thyroidectomy 20 years ago. No therapy for past 4 years. BMR -40%. Serum cholesterol 414 mg. %. (See also Figure 3.)	—	2	.97	.06-.07
19	Female, age 41, with hypothyroidism for 2 yrs. BMR -30 to to -40%. Serum cholesterol 500 mg. %.	1.0	1	.13	—
	<i>C. Hypopituitarism</i>				
20	Female, age 26, with the clinical picture of panhypopituitarism and with a history of hypoglycemic episodes.	—	1	.04	—
21	Female, age 61, with amenorrhea and myxedema following pregnancy 19 yrs. ago. Microcardia by x-ray. Cholesterol 625 mg. %. Serum Na 104 and Cl 73 meq/L. NPN 17 mg. %. Started on thyroid 5 days ago.	1.4	1	.95	—
22	Male, age 21, with dwarfism due to panhypopituitarism and with history of hypoglycemic attacks, one of which was brought on by thyroid therapy. FSH <2 M.U.	2.0	1	.09	—
23	Female, age 35, who had hypophyseal cyst removed 7 yrs. ago. Typical Addisonian crisis in association with appendiceal abscess 1 yr. ago with clear response to adrenal cortical extract therapy. FSH neg. at 6 and pos. at 3 M.U. BMR -30%.	1.6	1	.10	—
24	Male, age 65, who had pituitary tumor removed 10 yrs. ago. At present no gross clinical symptoms of hypopituitarism	3.6	1	.14	—
25	Male, age 44, who was dwarfed, obese and sexually infantile. Aspermatogenesis by testicular biopsy. BMR +5%. Normal insulin tolerance test. FSH 0 M.U.	2.0	1	.17	—
26	Male, age 18, who had craniopharyngioma removed 10 yrs. ago. At present dwarfed, slightly obese and sexually immature. Otherwise essentially symptom free. FSH pos. 3 M.U., neg. 6.5 M.U.	0.7	1	.29	—
	<i>D. Hyperadrenocorticism with Virilism</i>				
27	Female, age 16, with congenital virilism due to bilateral adrenal cortical hyperplasia proven by biopsy. No symptoms or signs of Cushing's syndrome or of hypoadrenocorticism.	28	2	.71	.23-.28
28	Female, age 12½, with congenital virilism presumably due to congenital adrenal cortical hyperplasia.	28	3	.25	.24-.29
29	Female, age 26, with acquired rather than congenital adrenal cortical virilism. Tallest in class until 10 yrs. old when she stopped growing and developed hirsutism. Presumably a case of bilateral adrenal cortical hyperplasia.	60 to 100	1	.57	—

TABLE I—(continued)

Patient Number	Description	17-KS	Urinary 11-OCS		
			No. of Determinations	Av.	Range
30	Female, age 5, with congenital virilism presumably due to adrenal cortical hyperplasia, large stature, heavy musculature, clitoral hypertrophy, pubic and axillary hirsutism.	8	1	.24	—
31	Male, age 8, who in early infancy developed disturbances of water and electrolyte metabolism, gastro-intestinal symptoms and skin pigmentation indistinguishable from those seen in Addison's disease. Has required NaCl and DOCA therapy since then. In early infancy also showed precocious masculine secondary sex development. At present marked precocity, pigmentation, normal insulin tolerance test. No evidence of Cushing's syndrome. Proven bilateral adrenal cortical hyperplasia. Hyperplastic adrenal cortical rests in testicular biopsy. No spermatogenesis.	± 200	3	.36	.15-.57
32	Female (Beth Israel Hosp.), age 3 yrs., with marked evidences of virilism, but without clear signs of Cushing's syndrome. Proven adrenal cortical carcinoma.	830	1	1.45	—
33	<i>E. Hyperadrenocorticism with Cushing's syndrome</i> Female, age 33, with characteristic full-blown Cushing's syndrome of 5 yrs. duration. No response to recent x-ray therapy to pituitary. See also Figure 4. Values given here are those obtained after therapy.	7.8 to 9.6	6	2.9	1.8-4.9
34	Female, age 20, with Cushing's syndrome. See also Figures 5 and 8 where effects of operation and of variations in protein intake are shown. At exploration adrenal hyperplasia was found.	14 to 26	4	.8	.18-1.5
35	Male, age 30, with active Cushing's syndrome. Left adrenal exploration revealed hyperplasia of that gland. See also Figure 5 for effect of operation on 11-OCS output.	10 to 24	7	1.15	.27-3.72
36	Female, age 38, with active Cushing's syndrome. Left adrenal exploration revealed hyperplasia of that gland. See also Figure 5 for effect of operation on 11-OCS output. Values shown here are pretreatment only.	15 to 18	2	.65	.27-1.02
37	Male, age 31, with active Cushing's syndrome.	23 to 44	4	1.53	.9-2.8
38	Male, age 30, with active Cushing's syndrome, untreated.	—	9	1.02	.65-1.46
39	Female, age 29 (Dr. R. Keating, Mayo Clinic) with characteristic full-blown Cushing's syndrome, untreated.	4.9	1	.89	—
40	Female, age 30 (Dr. J. Howard, Johns Hopkins), with active Cushing's. At operation bilateral adrenal hyperplasia found.	38 to 51	5	5.9	.5-12.0
41	Male, age 14 (Dr. L. Wilkins, Johns Hopkins), with active Cushing's syndrome. Biopsy of adrenal showed hyperplasia of the zone reticularis. See also Figure 8 for effects of variations in protein intake on 11-OCS output. Values shown here are for periods when patient was receiving diet of 2500 calories and 60 grams of protein.	—	3	2.95	1.4-3.7
42	Female, age 17 (Dr. L. Wilkins, Johns Hopkins), with active Cushing's syndrome. Skeleton markedly demineralized, patient completely bedridden. See also Figure 8 for effect of protein intake on 11-OCS output. Values shown here obtained while patient was receiving 1500 calories and 50 grams of protein daily.	—	2	.58	.52-.63

TABLE I—(continued)

Patient Number	Description	17-KS	Urinary 11-OCS		
			No. of Determinations	Av.	Range
43	Male, age 35 (Dr. Proger, Pratt Diagnostic), with gynecomastia, for 6 mos. Weight gain with loss of appetite, acne, purple striae, increased hirsutism, but no osteoporosis, hypertension or loss of potency. Adrenal cortical tumor removed. Value given was pre-operative. Postoperative 11-OCS values, 0.39 and 0.22 mg. per day.	—	1	1.3	—
44	Female, age 42, who developed active Cushing's syndrome under observation. Spine decalcified, decreased glucose tolerance. FSH <6.5 M.U.	3.8	<i>Date</i>		<i>Value</i>
			3/26/45		.36
			3/30/45		.34
			6/23/45		.34
			11/ 9/45		.75
			3/ 3/46		.79
			3/20/46		Adrenal tumor removed
45	<i>F. Simple Hirsutism</i> Female, age 35, with increased growth of hair over the entire body in past 3 years. Menstrual periods normal. No hypertrophy of clitoris. At laparotomy both adrenals appeared normal and biopsies of ovaries were normal. No evidence of Cushing's syndrome.	20.5	1	.32	—
46	Female, age 23, with apparently hereditary hirsutism. No clinical evidence of adrenal cortical virilism, Cushing's syndrome or other abnormalities.	13.2 to 23.6	1	.23	—
47	Female, age 28, with idiopathic hirsutism and obesity. Irregular menses. Exploration of adrenals negative. Adenofibroma of ovary removed.	8.2	1	.27	—
<i>G. Burn Patients</i>					
48	Male, age 38, with severe thermal burn. See Figure 6 for serial measurements.	—	16	.68	.35-1.2
49	Male, age 68, with extensive and fatal thermal burns. See Figure 6 for serial measurements.	—	6	.9	.4-1.7
50	Male, who was severely burned on the day of the assay and who died 5 days later.	—	1	1.1	—

The hospital record numbers of certain patients are as follows: the number in parenthesis is the patient's number in Table I and is followed by the initials of the hospital (MGH—Massachusetts General Hospital; PBBH—Peter Bent Brigham Hospital) and hospital number. Urine of the PBBH patients was obtained through the courtesy of Dr. George Thorn. (1) MGH 487800; (2) MGH 376861; (3) PBBH-F; (4) MGH 496895; (5) MGH 495076; (6) MGH 369416; (7) MGH 485500; (8) PBBH-KK; (9) PBBH-NX; (10) MGH 479500; (11) MGH 25029; (12) PBBH-JC; (13) PBBH-RG; (14) MGH 366564; (15) PBBH-JP; (16) PBBH-NM; (17) PBBH-MT; (18) MGH 474150; (19) MGH 488302; (21) MGH 488329; (22) MGH 64011; (23) MGH 46521; (24) MGH 486295;

TABLE I—(continued)

Patient Number	Description	17-KS	Urinary 11-OCS		
			No. of Determinations	Av.	Range
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32	Female (Beth Israel Hosp.), age 3 yrs., with marked evidences of virilism, but without clear signs of Cushing's syndrome. Proven adrenal cortical carcinoma.	830	1	1.45	—
33	<i>E. Hyperadrenocorticism with Cushing's syndrome</i> Female, age 33, with characteristic full-blown Cushing's syndrome of 5 yrs. duration. No response to recent x-ray therapy to pituitary. See also Figure 4. Values given here are those obtained after therapy.	7.8 to 9.6	6	2.9	1.8-4.9
34	Female, age 20, with Cushing's syndrome. See also Figures 5 and 8 where effects of operation and of variations in protein intake are shown. At exploration adrenal hyperplasia was found.	14 to 26	4	.8	.18-1.5
35	Male, age 30, with active Cushing's syndrome. Left adrenal exploration revealed hyperplasia of that gland. See also Figure 5 for effect of operation on 11-OCS output.	10 to 24	7	1.15	.27-3.72
36	Female, age 38, with active Cushing's syndrome. Left adrenal exploration revealed hyperplasia of that gland. See also Figure 5 for effect of operation on 11-OCS output. Values shown here are pretreatment only.	15 to 18	2	.65	.27-1.02
37	Male, age 31, with active Cushing's syndrome.	23 to 44	4	1.53	.9-2.8
38	Male, age 30, with active Cushing's syndrome, untreated.	—	9	1.02	.65-1.45
39	Female, age 29 (Dr. R. Keating, Mayo Clinic) with characteristic full-blown Cushing's syndrome, untreated.	4.9	1	.89	—
40	Female, age 30 (Dr. J. Howard, Johns Hopkins), with active Cushing's. At operation bilateral adrenal hyperplasia found.	38 to 51	5	5.9	.5-12.0
41	Male, age 14 (Dr. L. Wilkins, Johns Hopkins), with active Cushing's syndrome. Biopsy of adrenal showed hyperplasia of the zone reticularis. See also Figure 8 for effects of variations in protein intake on 11-OCS output. Values shown here are for periods when patient was receiving diet of 2500 calories and 60 grams of protein.	—	3	2.95	1.4-3.7
42	Female, age 17 (Dr. L. Wilkins, Johns Hopkins), with active Cushing's syndrome. Skeleton markedly demineralized, patient completely bedridden. See also Figure 8 for effect of protein intake on 11-OCS output. Values shown here obtained while patient was receiving 1500 calories and 50 grams of protein daily.	—	2	.58	.52-.63

TABLE I—(continued)

Patient Number	Description	17-KS	Urinary 11-OCS																						
			No. of Determinations	Av.	Range																				
43	Male, age 35 (Dr. Proger, Pratt Diagnostic), with gynecomastia, for 6 mos. Weight gain with loss of appetite, acne, purple striae, increased hirsutism, but no osteoporosis, hypertension or loss of potency. Adrenal cortical tumor removed. Value given was pre-operative. Postoperative 11-OCS values, 0.39 and 0.22 mg. per day.	—	1	1.3	—																				
44	Female, age 42, who developed active Cushing's syndrome under observation. Spine decalcified, decreased glucose tolerance, FSH <6.5 M.U.	3.8	<table><thead><tr><th>Date</th><th>Value</th></tr></thead><tbody><tr><td>3/26/45</td><td>.36</td></tr><tr><td>3/30/45</td><td>.34</td></tr><tr><td>6/23/45</td><td>.34</td></tr><tr><td>11/ 9/45</td><td>.75</td></tr><tr><td>3/ 3/46</td><td>.79</td></tr><tr><td>3/20/46</td><td>Adrenal tumor removed</td></tr><tr><td>3/27/46</td><td>.28</td></tr><tr><td>4/ 5/46</td><td>.16</td></tr><tr><td>6/20/46</td><td>.30</td></tr></tbody></table>	Date	Value	3/26/45	.36	3/30/45	.34	6/23/45	.34	11/ 9/45	.75	3/ 3/46	.79	3/20/46	Adrenal tumor removed	3/27/46	.28	4/ 5/46	.16	6/20/46	.30		
Date	Value																								
3/26/45	.36																								
3/30/45	.34																								
6/23/45	.34																								
11/ 9/45	.75																								
3/ 3/46	.79																								
3/20/46	Adrenal tumor removed																								
3/27/46	.28																								
4/ 5/46	.16																								
6/20/46	.30																								
45	<i>F. Simple Hirsutism</i> Female, age 35, with increased growth of hair over the entire body in past 3 years. Menstrual periods normal. No hypertrophy of clitoris. At laparotomy both adrenals appeared normal and biopsies of ovaries were normal. No evidence of Cushing's syndrome.	20.5	1	.32	—																				
46	Female, age 23, with apparently hereditary hirsutism. No clinical evidence of adrenal cortical virilism, Cushing's syndrome or other abnormalities.	13.2 to 23.6	1	.23	—																				
47	Female, age 28, with idiopathic hirsutism and obesity. Irregular menses. Exploration of adrenals negative. Adenofibroma of ovary removed.	8.2	1	.27	—																				
48	<i>G. Burn Patients</i> Male, age 38, with severe thermal burn. See Figure 6 for serial measurements.	—	16	.68	.38-1.2																				
49	Male, age 68, with extensive and fatal thermal burns. See Figure 6 for serial measurements.	—	6	.9	.4-1.7																				
50	Male, who was severely burned on the day of the assay and who died 5 days later.	—	1	1.1	—																				

The hospital record numbers of certain patients are as follows: the number in parenthesis is the patient's number in Table I and is followed by the initials of the hospital (MGH—Massachusetts General Hospital; PBBH—Peter Bent Brigham Hospital) and hospital number. Urine of the PBBH patients was obtained through the courtesy of Dr. George Thorn. (1) MGH 487800; (2) MGH 376861; (3) PBBH-F; (4) MGH 496895; (5) MGH 495076; (6) MGH 369416; (7) MGH 485500; (8) PBBH-KK; (9) PBBH-MN; (10) MGH 479500; (11) MGH 25029; (12) PBBH-JC; (13) PBBH-RG; (14) MGH 366564; (15) PBBH-JP; (16) PBBH-NM; (17) PBBH-MT; (18) MGH 474150; (19) MGH 488302; (21) MGH 488329; (22) MGH 64011; (23) MGH 46521; (24) MGH 486295;

(25) MGH 218519; (26) MGH 350596; (27) MGH 354826; (28) MGH 169536; (31) MGH 366532; (33) MGH 467276; (34) MGH 499922; (35) MGH 511658; (36) MGH 527071; (37) MGH 526033; (44) MGH 484185; (47) MGH 42182; (48) MGH 491023; (49) MGH 481638; (50) MGH 482818; (51) MGH 430664.

roughly 100 times the expected normal value for a child her age, the 11-OCS value was only about four to five times the approximate normal for young children.

Of exceptional interest were the values obtained for patient No. 31 who presented clinical evidences of a remarkable dissociation of adrenal cortical function. The marked disturbances in water and electrolyte metabolism and their response to desoxycorticosterone acetate therapy suggested that the adrenals were failing to produce a normal quota of that type of hormone. Thus it seemed probable that in this patient the urinary 17-KS (markedly elevated) and the 11-OCS (normal to slightly elevated) were not derived from adrenal hormones with the desoxycorticosterone type of metabolic action. The stable carbohydrate metabolism, including a normal ability to tolerate 0.1 unit of regular insulin per kilogram of body weight given intravenously (5), indicated that he was probably not suffering from a deficiency of the adrenal cortical carbohydrate or "S" hormone, whereas the absence of signs of Cushing's syndrome ruled against an excess of this type of agent (1). The essentially normal 11-OCS value corresponds satisfactorily with these clinical findings. Finally, the marked masculine precocity indicated the presence of excess androgens which were almost certainly of adrenal cortical origin. The evidence of the literature shows that the urinary 17-KS are excretory transformation products and hence an index of the rate of production of adrenal cortical (and testicular) androgens (7). Hence the markedly elevated 17-KS output was consistent with this portion of the clinical picture.

*Hyperadrenocorticism with Cushing's Syndrome.* Section E, Table I, presents observations on 12 patients with this condition. With the partial exception of patient No. 34 all of these patients were in an active phase of the disease. Except where indicated they were not under treatment at the time the measurements were made. It is seen that there was a distinct tendency towards abnormally elevated 11-OCS values (average 1.7 mg., range 0.6 to 12.0 mg. per day). Roughly speaking the highest values were obtained for patients suffering most severely from the disease. In this connection it is of interest that values obtained on 3 other patients whose Cushing's syndrome was considered on clinical grounds to be in remission were within normal limits (average .28 mg., range .21 to .38 mg. per day). Figure 4 also illustrates fluctuations in the 11-OCS output induced by the

administration and withdrawal of testosterone propionate to patient No. 33. When the drug was given the 11-OCS values tended to fall, while they rose when it was stopped. Removal of a tumor of the right adrenal cortex resulted in an abrupt drop in the 11-OCS to a low normal level. This observation suggests strongly that the 11-OCS appearing in the urine of this patient originated in the adrenal cortical tumor.

In contrast to the patients with adrenal cortical virilism, the urinary

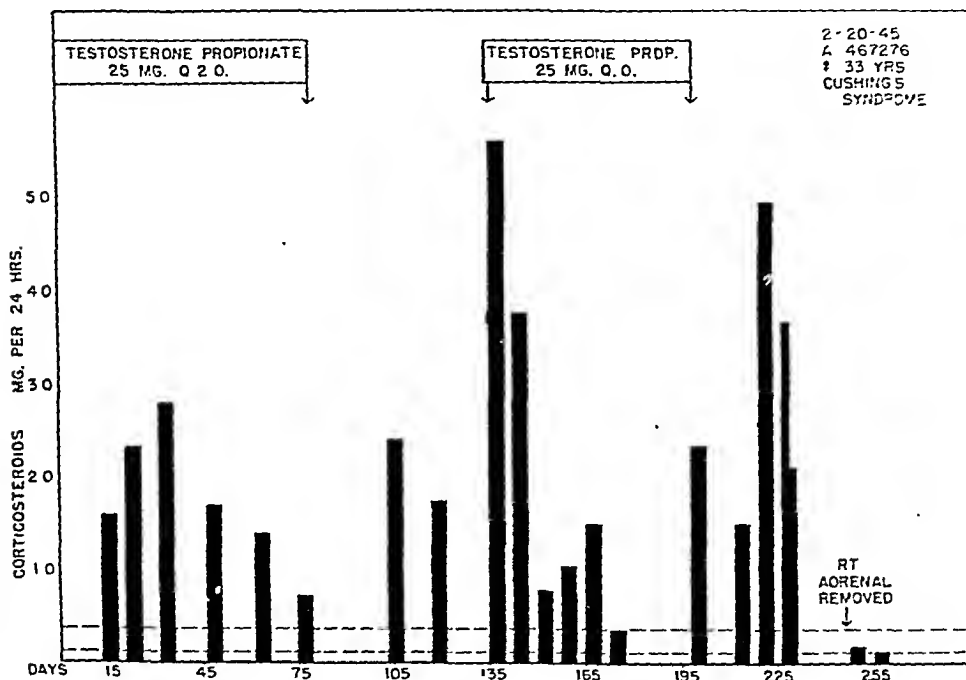


FIG. 4. Effect of testosterone therapy and of the removal of an adrenal cortical tumor upon the 11-OCS values of a patient with active Cushing's syndrome (No. 33, Table I). The interrupted horizontal lines correspond to those of Figure 1.

17-KS output was proportionately much less elevated in the Cushing's syndrome patients than was the 11-OCS output.

*Simple Hirsutism.* Because patients with hyperadrenocorticism and either virilism or Cushing's syndrome tend to have abnormal hirsutism, it was of interest to obtain a few measurements on patients with hirsutism not apparently due to adrenal cortical disease. The excretion of 11-OCS by these patients (Section F, Table I) was not abnormal. The 17-KS values of 2 of the 3 patients were slightly elevated.



*Effect of Trauma upon 11-OCS Output (Alarm Reaction).* Figure 5 presents observations on the urinary excretion of 11-OCS by 3 of the patients with Cushing's syndrome (Nos. 34, 35 and 36) before and after surgical exploration of the adrenal gland. In all of these patients hyperplasia of the adrenal gland was noted, but except for a small specimen for biopsy the gland was not removed. It can be seen that the output of 11-OCS rose

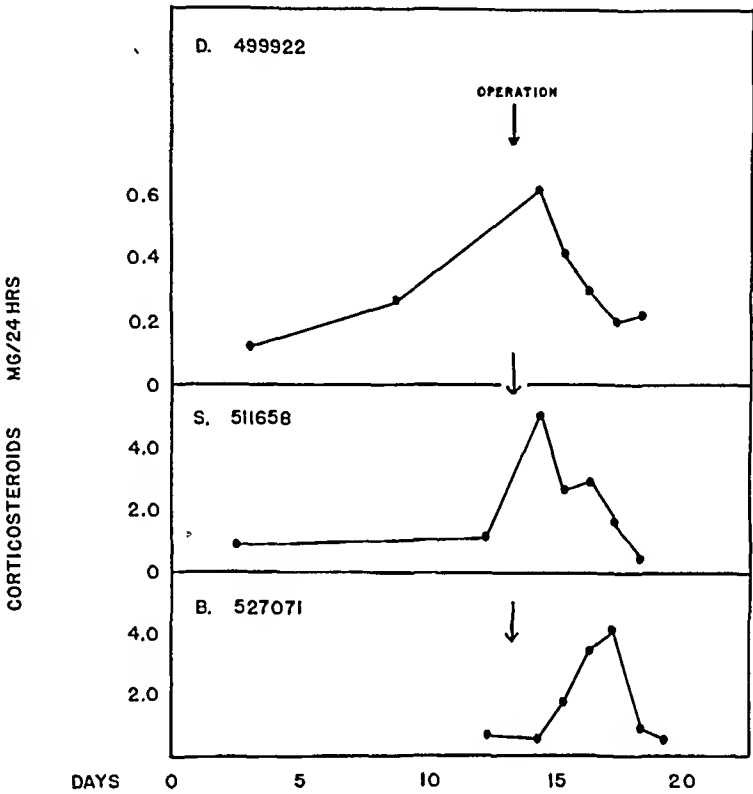


FIG. 5. Excretion of 11-OCS by 3 patients (Nos. 34 to 36, Table I) with Cushing's syndrome pre- and postsurgical exploration of an adrenal gland.

appreciably within one to four days post-operatively and then subsided to essentially preoperative levels.

Figure 6, on the other hand, shows the 11-OCS values obtained on 2 subjects (Section G, Table I) who suffered extensive burns. The patient whose course is illustrated in the left hand section of the figure was followed for 60 days following the burn. Throughout the major portion of this period the 11-OCS output was abnormally elevated. Two peaks of excretion are evident. The first which attained a maximum at about the 20th post-burn day was apparently related to the initial burn; the second peak (38th day) occurred in association with anesthesia and skin grafting. The terminal

subsidence of 11-OCS excretion values to normal levels coincided with essentially complete healing of the skin and with the clinical recovery of the patient. While the 17-KS values remained within normal limits throughout the period of observation, they tended to vary in the opposite direction from the 11-OCS. In the second patient of Figure 6 (right hand

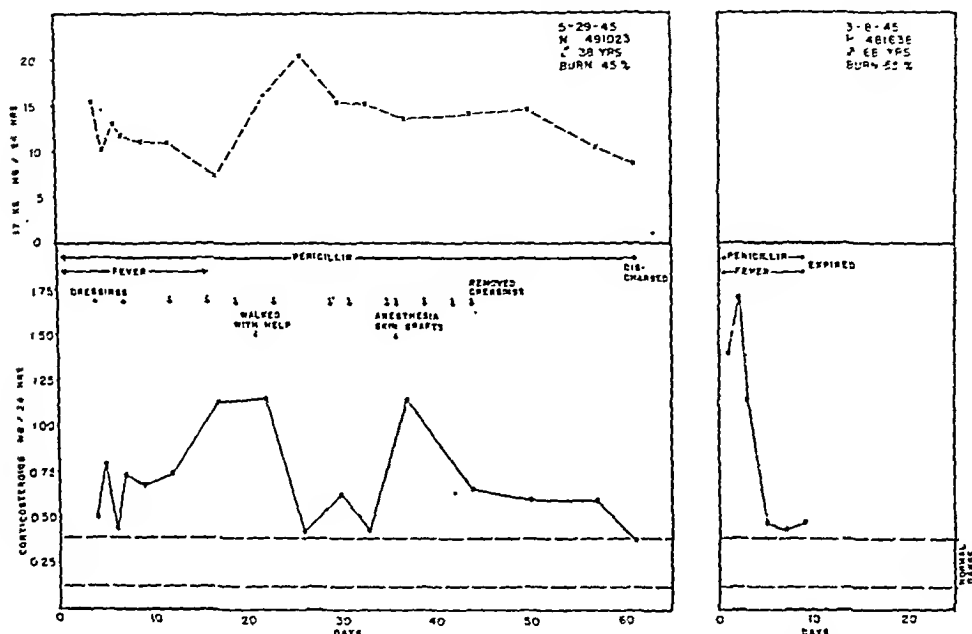


FIG. 6. Excretion of 11-OCS and of 17-KS by patients (Nos. 48, 49, Table I) following extensive burns. Day zero was the day of the burn. The interrupted horizontal lines correspond to those of Figure 1.

section), there was a marked elevation in the output of 11-OCS shortly after the burn occurred, followed by an abrupt fall almost to normal levels. The patient died on the ninth day. In a third patient (No. 50, Table I) there also was an elevated 11-OCS output on the day of the burn.

*Nitrogen (Protein, Amino Acids or Plasma) Intake and 11-OCS Output.* There are recorded in the literature observations suggesting that a high protein intake prompts hypertrophy of the adrenal cortex (2, 6). Accordingly, the possible effects of variations in the intake of nitrogenous nutrients were studied in a variety of subjects (Figs. 7 and 8).

Figure 7 gives data on normal subjects and on 2 patients with idiopathic osteoporosis. In the normal subject C, the excretion of 11-OCS during periods when an average *ad lib.* diet was eaten (left hand and right

hand sections of top left hand diagram) was within normal limits. The middle section of that diagram shows that the subject ate isocaloric diets ( $\pm 4000$  calories per day) containing, respectively, 31, 39 and 9 Gm. of protein nitrogen per day. Stated in other words the two high-protein diets

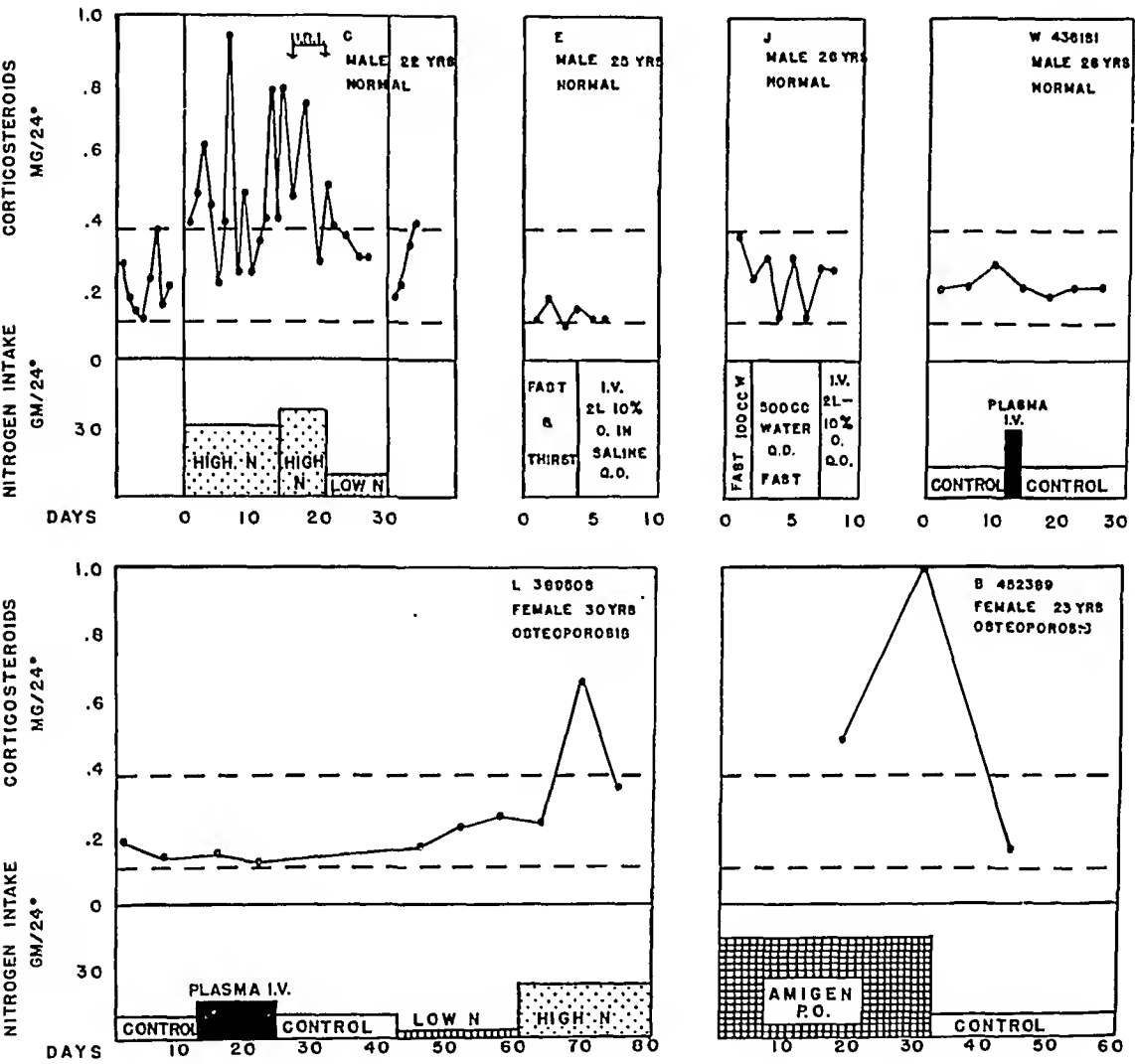


FIG. 7. Relation between protein intake and urinary 11-OCS excretion by normal subjects and by 2 patients with idiopathic osteoporosis. See text for detailed description. The interrupted horizontal lines correspond to those of previous charts.

provided 3.0 and 3.8 Gm. and the low-protein diet 0.8 Gm. protein per kilogram of body weight per day. While the subject was taking the higher protein diets there was a definite tendency toward moderately elevated 11-OCS values. At other times the output of 11-OCS was within normal limits. Normal subjects E and J (center, top row, Fig. 7) show that a zero

protein intake with or without fasting and thirsting had no appreciable effect upon the 11-OCS excretion. In the case of the normal male, W (top right hand diagram), the intravenous administration of 1500 cc. of plasma

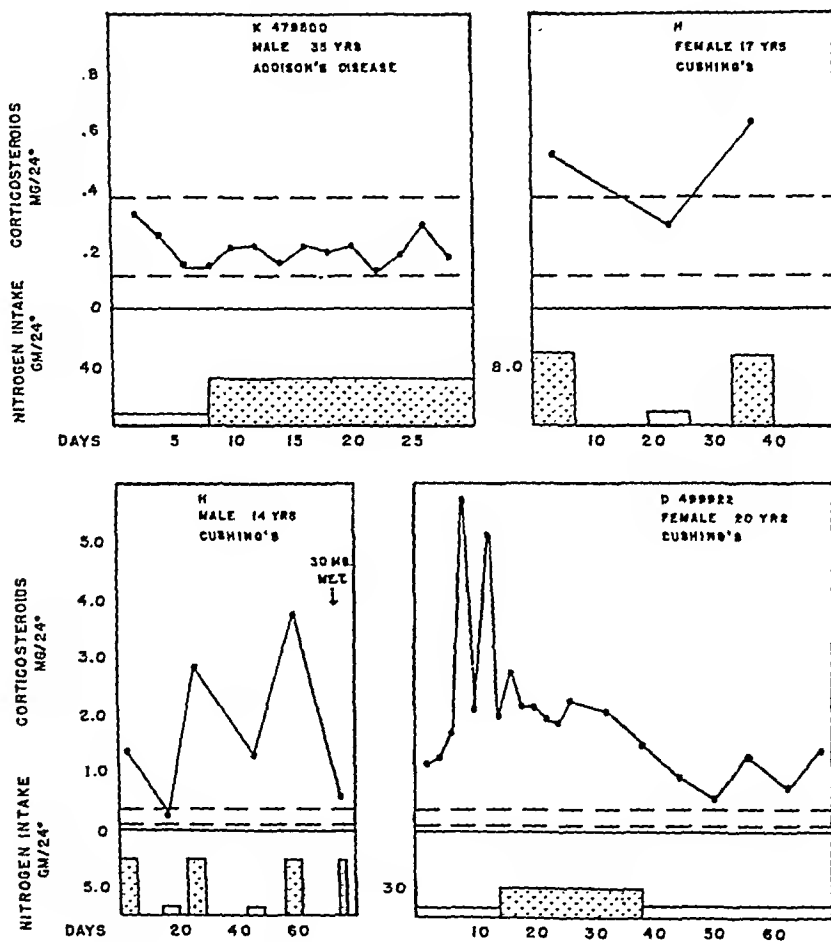


FIG. 8. Relation between protein intake and 11-OCS output by patients with adrenal cortical disease. See text for detailed description. The interrupted horizontal lines correspond to those of previous charts. Patient K is No. 10, female patient H is No. 43, male patient H is No. 42, and patient D is No. 35, Table I.

(194 Gm. of protein) daily for two days was also without effect on the 11-OCS excretion level. The same lack of response was evident in the osteoporotic patient, L (lower left diagram), during the period when she received 500 cc. of plasma intravenously daily for 12 days. On the other

hand, the ingestion of a relatively high protein diet (3.0 Gm. per kilogram of body weight per day) by this patient resulted in an apparent rise in 11-OCS output. Her total caloric intake was maintained constantly at about 1800 calories per day. A similar and more striking rise was observed in patient B (lower right hand diagram) when she ate 5.9 Gm. of amino acids per kilogram of body weight daily for 34 days. In this patient also the total caloric intake was constantly kept at about 1750 calories per day.

Because these data suggested that a high oral protein intake prompted a rise in 11-OCS values and hence presumably in adrenal cortical activity, it seemed of interest to study the effect of variations in protein intake on patients with adrenal cortical disease. It might be expected that a patient with hypoadrenocorticism would be unable to respond to this type of stimulus by increasing the 11-OCS output. This idea appears to be borne out by the findings on patient K (top left diagram, Fig. 8) who showed no elevation in 11-OCS values when the protein content of his daily diet was increased from 0.5 to 3.0 Gm. per kilogram of body weight. Two patients with Cushing's syndrome (patients H and H, respectively, top right and lower left diagrams of Fig. 8), however, tended to show a marked increase in 11-OCS values on a high protein diet and a decrease in 11-OCS values on a low protein diet. Incidentally, it is noteworthy that the administration of methyl testosterone to male patient H (lower left diagram) apparently counteracted the effects of the high protein intake, so that when the patient was receiving both the relatively high protein diet and the drug, the value obtained was only slightly above normal limits. In contrast to the foregoing are observations on patient D, who also had active Cushing's syndrome (lower right diagram, Fig. 8). In this patient variations in protein intake bore no clear relation to 11-OCS values. No explanation for this discrepancy is at hand. It therefore appears that the tendency noted in the other subjects for the 11-OCS output to rise with a high protein intake was an elective rather than an obligatory functional response.

*Relation Between the Administration of Various Steroid Hormones and Related Substances to the 11-OCS Output.* Table II records data showing the effects of a variety of these agents upon the 11-OCS excretion levels of 2 patients, one of whom had adrenal cortical virilism and the other postmenopausal osteoporosis and Paget's disease. In both patients the respective control values were within normal limits. While small variations in 11-OCS values were noted, careful study failed to reveal any clear relation between these variations and the respective therapy given.

Not shown in the table are a series of observations on 8 patients with

Addison's disease under the care of Dr. G. Thorn at the Peter Bent Brigham Hospital, Boston. In each of these the urinary 11-OCS output was measured both before and after approximately three days of corticosterone treatment (15 to 60 mg.<sup>2</sup> intramuscularly daily). No consistent or significant difference between control and treatment period values was evident. That

TABLE II

Patient Number	Diagnosis	Treatment	Daily Dose	No. of 6-day periods	11-OCS	
					Av.	Range
28	Adrenal Cortical Virilism	None	mg. —	3	mg. .25	mg. .24-.29
		Desoxycorticosterone acetate	20	1	.44	—
		Desoxycorticosterone acetate	30	1	.26	—
		Pregnanelone	30	2	.30	.30-.30
		Pregnanelone	60	1	.19	—
		Progesterone	25	1	.16	—
		Progesterone	75	1	.17	—
		Progesterone } Stilbestrol }	75 3	5	.36	.19-.52
		Ethyl testosterone	20	6	.25	.15-.45
		Methyl testosterone	50	3	.33	.20-.41
51	Female, Age 53, Postmenopausal Osteoporosis and Paget's Disease	None	—	4	.26	.10-.34
		Stilbestrol	1	5	.18	.10-.26
		Stilbestrol	15	5	.19	.10-.24
		Stilbestrol } Progesterone }	15 25	2	.18	.13-.23
		Stilbestrol } Progesterone }	15 100	1	.29	—
		Stilbestrol	15	2	.21	.18-.24

is, there was no evidence that administered corticosterone was recovered from the urine by the present analytic procedure. The same appeared to be true for desoxycorticosterone (Table II and unpublished observations). These findings are not too surprising for it was learned while devising the assay method that only corticosteroids with an oxygen at carbon 11 and an hydroxyl group at carbon 17 can be recovered quantitatively (8). Corticosterone lacks one and desoxycorticosterone lacks both of these characteristics.

On the other hand, it will be remembered that methyl testosterone

therapy was followed by an appreciable fall in the 11-OCS output by two patients with active Cushing's syndrome (patients No. 33 and 42, Table I and Figs. 4 and 8). This finding is in keeping with similar observations carried out with the aid of a biologic method for assaying urinary corticoids (10).

## COMMENTS AND SUMMARY

I. This study consists of an analysis of the urinary "11-oxycorticosteroid" (11-OCS) excretion in normal individuals and in patients with certain endocrinopathies.

II. The procedure, previously reported (8), consists of a colorimetric assay of the adrenal cortical "sugar hormone" or "S hormone"; the value obtained is thought to be an index to those 21 carbon adrenal-cortical steroids which have a ketone or hydroxyl group on the eleventh carbon atom and an hydroxyl group on the 17th carbon atom.

III. The average 24-hour excretion obtained for 11-OCS in normal young adults was 0.22 milligram with a range from 0.10 to 0.44 milligram.

IV. The average value obtained for 17 patients with panhypoadrenocorticism (Addison's Disease) was 0.14 milligram with a range of 0.02 to 0.29 milligram; evidence is presented which indicates that the overlapping between normal individuals and patients with Addison's disease in their assay values would be minimal if determinations were carried out on more than one 24-hour urinary specimen; the administration of 11-desoxycorticosterone acetate had no effect on the assay values of patients with Addison's disease.

V. The 11-OCS assay was less than 0.10 milligram per 24 hours in 4 of 7 patients with pan-hypopituitarism and less than 0.18 milligram in 6 of the 7.

VI. The 11-OCS assays of two patients with hypothyroidism, a condition in which clinically there is no evidence of "sugar hormone" lack, were below 0.1 milligram per 24 hours; in one of these patients, on whom the effect of thyroid medication was studied, the assay value rose to normal; it is suggested that the low value in this disease may be on a compensatory basis.

VII. In five cases of hyperadrenocorticism-with-respect-to-the-N-hormone (adrenal virilism) with high 17-ketosteroid excretions the 11-OCS assay was normal or only slightly elevated; in one case where the hyperadrenocorticism-with-respect-to-the-N-hormone was the result of adrenal cortical neoplasm the 11-OCS excretion was high (1.45 milligrams per 24 hours).

VIII. Of exceptional interest was one of the five patients mentioned

above, a male child with hyperadrenocorticism with virilism; this individual exhibited hyperadrenocorticism-with-respect-to-the-N-hormone as judged by virilism and 17-ketosteroid excretion, hypoadrenocorticism-with-respect-to-the-"salt-and-water"-hormone as judged by electrolyte studies and response to desoxycorticosterone acetate, and iso-adrenocorticism-with-respect-to-the-S-hormone as judged by carbohydrate studies and 11-OCS excretions.

IX. In 12 patients with hyperadrenocorticism-with-respect-to-the-S-hormone (Cushing's Syndrome) the 11-OCS assay averaged 1.7 milligrams with a range of 0.6 to 12.0 milligrams per 24 hours; the 11-OCS excretions in three other cases thought to be in remission were within normal limits.

X. Non-specific trauma ("Alarm Reaction") resulted in a rise of urinary 11-OCS assays; thus, values of the order of magnitude found in Cushing's syndrome were obtained in three patients with severe burns, the excretion falling to normal in one of these patients, interestingly enough, about three days before a fatal issue; moreover, exploration of the adrenal glands in three cases of Cushing's syndrome in which no tumors were found likewise resulted in a temporary rise of urinary 11-OCS output.

XI. In experiments on normal subjects or patients without adrenal cortical dysfunction the urinary 11-OCS assays were increased by a high oral protein intake or by oral casein hydrolyzate but were unaffected by plasma given intravenously or by starvation with or without thirsting; these observations, however, are quite fragmentary.

XII. In a patient with pan-hypoadrenocorticism the 11-OCS urinary assay was unaffected by a high protein diet; in two of three patients with Cushing's Syndrome it was repeatedly elevated by a high protein diet.

XIII. The high urinary 11-OCS values in Cushing's Syndrome (two cases) were decreased by testosterone therapy; this is in agreement with the unpublished observations of Deane and Greep (4) that testosterone administration to rats tends to decrease the steroid content of the zona fasciculata where it is thought the 11-OCS are produced (3, 9).

XIV. It is tentatively concluded that the 11-OCS output as measured here may be taken as an index of the rate of production of the carbohydrate-regulating hormones (gluconeogenic or sugar hormone) of the adrenal cortex and that as such they give distinctly different information from that provided by the urinary 17-ketosteroid measurement.

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# THE PREGNANDIOL PRECIPITATION TEST

## CLINICAL APPLICATION OF A RAPID METHOD FOR THE DIAGNOSIS OF PREGNANCY\*

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REPEATED attempts have been made to perfect a chemical test for the early diagnosis of pregnancy through identification of pregnandiol in the urine of amenorrhoeic women (4, 7, 10). Technical difficulties have hindered extensive adoption of the various methods which have been proposed; conflicting reports have been published concerning their practical usefulness. A new and greatly simplified technic has recently been developed in our laboratory (9). Sufficient data have now been accumulated to permit a partial evaluation of the clinical usefulness of this procedure.

### PREGNANDIOL PRECIPITATION TEST

This test is an outgrowth of our investigations of the Astwood and Jones (1) and Guterman (5) procedures. While performing a Guterman test in a suspected case of ectopic pregnancy, a macroscopic precipitate was noted at one of the intermediate steps of the technic. A diagnosis of presumptive pregnancy based upon this end-point was substantiated at operation; a positive Guterman-color reaction and a positive Friedman test were subsequently obtained from the same urine specimen. Encouraged by this observation, a technic for the precipitation of unpurified pregnandiol was developed which eliminated the last five steps of Guterman's procedure. The time required for this technic is less than two hours.

### Technic

#### A. Hydrolysis and extraction of pregnandiol.

1. Secure a first morning urine specimen and record specific gravity.
2. Measure 100 ml. of urine and pour into a 500 ml. Erlenmeyer flask.
3. Add 50 ml. of toluene (Reagent quality), 10 ml. of concentrated hydrochloric acid, and two glass beads to the flask.
4. Connect the flask to a vertical Liebig condenser and allow the sample to reflux on the hot plate for 15 minutes.
5. Remove the flask and cool rapidly to room temperature under a

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water tap. Avoid agitation of the sample while cooling to prevent formation of emulsion.

6. Transfer the sample to a 500 ml. separatory funnel; draw off and discard the lower layer (urine).
7. Small amounts of emulsion present at this point can be disregarded. If large amounts are present, transfer the sample to a 100 ml. tube and centrifuge for 5 minutes. The toluene and associated urine is then poured into the separatory funnel leaving the emulsion in the tube.
8. Add 15 ml. of 0.1 N sodium hydroxide to the separatory funnel and wash by inverting twice and swirling the sample gently.
9. The sodium hydroxide settles to the bottom of the funnel and is discarded.
10. Repeat step (8) with an additional 15 ml. of sodium hydroxide and follow the same procedure with two 15 ml. portions of water.
11. After the last washing with water, make a very careful separation so that only the toluene layer is transferred to a *dry* 125 ml. Erlenmeyer flask.
12. Allow the sample to stand undisturbed for at least 5 minutes; then pour into a second *dry* 125 ml. Erlenmeyer flask, making certain that any droplets of water which may have collected are left behind.

#### B. Precipitation of impurities.

13. Add 2 glass beads and bring the sample to boiling on the hot plate.
14. When the vapors reach the mouth of the flask, slowly add 10 ml. of 2% sodium hydroxide in absolute methanol while the flask remains on the hot plate.
15. Boil until the methanol has evaporated and the toluene is reduced to about one-half its original volume.
16. Filter, while hot, through a fritted glass filter (Pyrex, medium porosity) using suction. Collect the filtrate in a large test tube.
17. Add 15 ml. of toluene to the flask as a rinse, heat to boiling, and use it to wash the precipitate remaining on the filter.
18. Pour the filtrate from the test tube into a dry 125 ml. Erlenmeyer flask and take to dryness on the hot plate. Use gentle air stream to remove the last traces of toluene.

#### C. Precipitation of pregnandiol.

19. Add 5 ml. of acetone to the residue in the flask.
20. Place flask containing acetone on the hot plate. When it begins to

boil, add 25 ml. of boiling 0.1 N sodium hydroxide slowly, 3-4 ml. at a time.

21. Remove the sample from the hot plate.

#### D. Reading of result.

22. Swirl the sample and observe the side of the flask above the level of the liquid, standing in front of a window or good source of light. In samples containing 0.75 mg. or more, of pregnandiol, a precipitate is evident immediately. Due to surface tension of the liquid and the lightness of the precipitate, it is carried up the side of the flask and becomes evident as tiny, white particles.
23. If no precipitate is immediately evident, place the sample in an ice water bath for ten minutes. At the end of this time, remove from ice bath and allow to stand for 30 minutes at room temperature. Again observe as in (22).
24. As a method of checking the amount and character of the precipitate we have recently followed the procedure used at the Harper Hospital Clinical Laboratory for reading and grading Kahn tests. One cc. of the sample is placed in a standard Kahn tube and viewed in a fluorescent light box. The reaction can thus be graded as 0, x, xx, xxx, xxxx.

#### E. Description of precipitate.

The substance which is precipitated under the conditions listed above is manifestly impure pregnandiol, contaminated by cholesterol and other steroids. The precipitate is birefringent and does not form clearly-defined crystals.

#### CONTROL STUDIES

To test the efficiency of this method, preliminary studies were carried out before subjecting the method to clinical trial, as follows: (a) recovery tests of weighed amount of crystalline pregnandiol added to pooled samples of male urine, (b) determinations in normal pregnancy and (c) non-pregnant states.

(a) **Crystalline pregnandiol.** As stated in our original publication (9), weighed amounts of pure pregnandiol were added to 100 cc. aliquots of pooled samples of male urine. Positive pregnandiol precipitation was obtained consistently in samples containing 0.50-0.75 mg. of pregnandiol. Roughly quantitative estimates of the precipitate could be obtained by centrifugation of the final sample in a graduated tube, as shown in Fig. 1. The highly variable amounts of precipitate observed during normal preg-

nancy and during the luteal phase of the normal cycle are shown in Fig. 2.

(b) **Normal pregnancy.** First morning samples from 42 clinic patients known to have been normally pregnant were tested from the first to the tenth months of gestation. All showed positive pregnandiol precipitation which, however, varied greatly in intensity; very strongly positive reactions, manifested by abundant, flocculent precipitate, were observed most frequently during the second and third trimesters. This is in keeping with the observation of Venning and Browne (3) that pregnandiol excretion increases from the fourth month to term. These results are summarized in Table I:

TABLE I. KNOWN PREGNANT CONTROLS

Stage of Gestation	Pregnandiol Positive	Pregnandiol Negative
First trimester	5	0
Second trimester	13	0
Third trimester	24	0

Pregnandiol excretion during labor and early puerperium was studied in 9 patients following normal pregnancy and delivery, and showed the early disappearance of pregnandiol from the urine post-partum:

Patient	Pregnandiol Tests						
	Labor	Days post-partum					
		1	2	3	4	5	6
#1	xx	—	—	—			
#2	xx	xx	xx	xx	x		
#3	xx	xx	xx	—	—	—	
#4	xx	—	—	—			
#5	xx	x	x	—	—		
#6	xx	—	—	—			
#7	xx	—	—				
#8	xx	x	—	—			
#9	xx		xx	x	x	x	

xx strongly positive pregnandiol precipitate

x positive pregnandiol precipitate

— negative pregnandiol test

(c) **Non-pregnant controls.** The pregnandiol precipitation test was carried out in 52 non-pregnant subjects (5 normal males; 47 women). As is

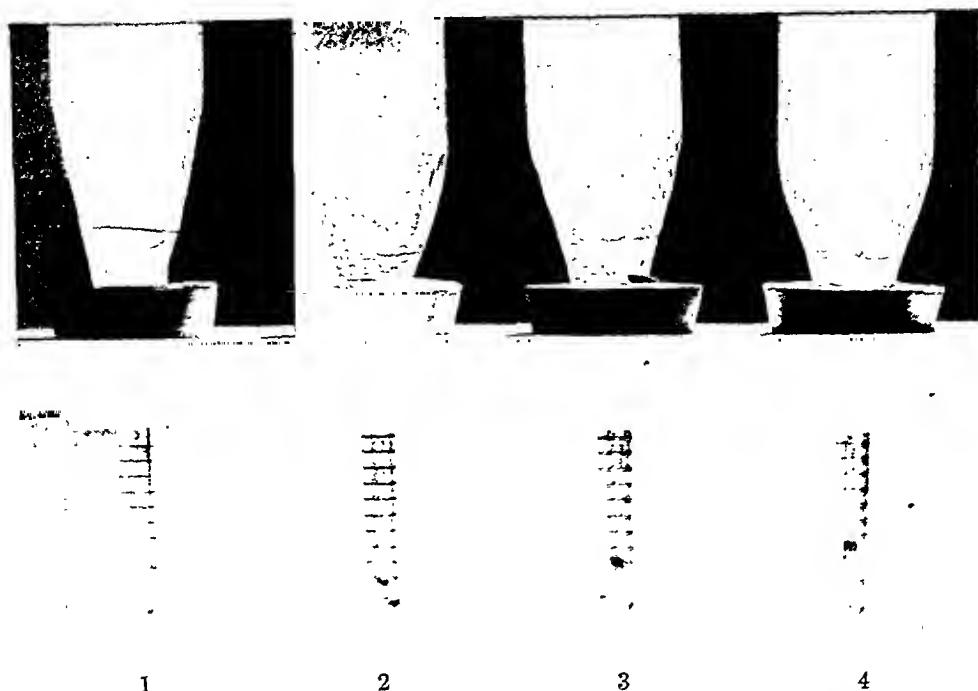


FIG. 1. Pregnandiol precipitation test showing recovery after addition of pregnandiol to male urine. #1 0.0 mg. pregnandiol added to 100 ml. male urine. #2. 1.0 mg. pregnandiol added to 100 ml. male urine. #3. 2.0 mg. pregnandiol added to 100 ml. male urine. #4. 3.0 mg. pregnandiol added to 100 ml. male urine.

TABLE II. NON-PREGNANT CONTROLS

No. Patients	Clinical Diagnosis	Pregnandiol Positive	Pregnandiol Negative
5	Normal males	0	5
8	Normally menstruating women		
	15th day of cycle	0	8
	16th-26th day of cycle	33	2
11	Prolonged secondary amenorrhea	0	11
3	Lactation amenorrhea	0	3
3	Late menopause	0	3
2	Delayed menses (3 days)	0	2
1	Postmenopausal bleeding	0	1
1	Menorrhagia	0	1
2	Uterine fibroids	0	2
1	Endometriosis	0	1
15	Miscellaneous medical conditions	0	15
52			

shown in Table II; positive pregnandiol tests in non-pregnant women were observed only during the luteal phase of the normal menstrual cycle. In all other subjects of the control series, negative results were obtained. Six normally menstruating women, tested daily during the luteal phases of 24

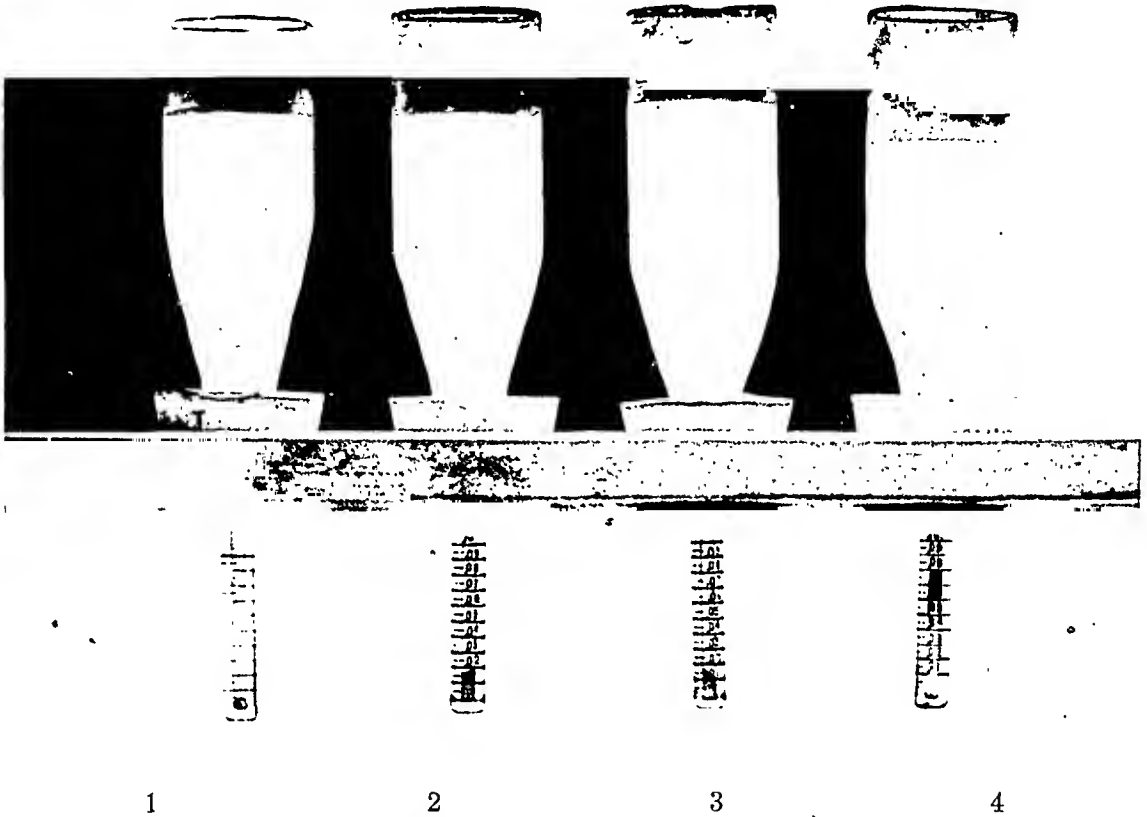


FIG. 2. Pregnandiol precipitation test in normal pregnancy and in non-pregnant states. #1. Twenty-first day of normal cycle. M.M. Para 0. Gravida 0. Age 22. #2 Normal pregnancy—8 weeks. E.L. Para 0. Gravida 1. Age 23. #3. Twenty-third day of normal cycle. R.P. Para 2. Gravida 2. Age 27. #4. Normal pregnancy—6 weeks. W.W. Para 1. Gravida 3. Age 25.

cycles, showed pregnandiol excretion on one or more days of each cycle; each of 2 normally menstruating women tested similarly during a total of 11 cycles showed no pregnandiol excretion throughout one cycle. Further studies are in progress to determine whether this lack of pregnandiol excretion can be related to anovulatory menstruation. The duration of positive pregnandiol tests varied greatly in this group, as did also the intensity of the reaction.

Repeated tests in 11 patients with prolonged secondary amenorrhea were consistently negative. These findings support the views of Buxton (4),

Hain and Robertson (7), Wilson and Randall (10) and others that *pregnandiol excretion in the presence of amenorrhea in otherwise normally and regularly menstruating women is presumptive evidence of pregnancy*. As pointed out by Morrow and Benua (8), false positive pregnancy tests may be expected in instances of late ovulation with resulting prolongation of the cycle so that corpus luteum activity may be at its peak when the usual bleeding period has been passed.

### CLINICAL APPLICATIONS

The diagnostic value of the pregnandiol precipitation test was next judged from 121 routine samples submitted to the laboratory for the diagnosis of pregnancy. Simultaneous Friedman reactions, performed in all but one instance, permitted a comparison of the accuracy of these two tests. Additional studies were made of 1 case of hydatidiform mole, with negative pregnandiol and positive Aschheim-Zondek test and 14 cases of threatened abortion. The latter study was carried out to test Guterman's claim (6) that the fate of threatened abortion can be predicted with accuracy by pregnandiol excretion levels during the period of symptoms.

**Normal pregnancy.** Correct positive pregnandiol tests were obtained in 65 of 67 patients who, on subsequent follow-up were found to have been normally pregnant; the Friedman test was correct in 66 of 67 instances. These results are summarized in Table III:

TABLE III. NORMAL PREGNANCY

Cases	Amenorrhea	Pregnandiol Test		Friedman Test	
		Correct positive	False negative	Correct positive	False negative
8	1 week	8	0	7	1
6	2 weeks	5	1	6	0
4	3 weeks	4	0	4	0
7	4 weeks	7	0	7	0
19	Over 4 weeks	18	1	19	0
23	Unknown	23	0	23	0
—	—	—	—	—	—
67		65	2	66	1

The earliest positive diagnoses of pregnancy were made during the first week of amenorrhea. Serial determinations of pregnandiol excretion from the luteal phase of the cycle through the early phases of amenorrhea permitted a presumptive diagnosis prior to the 35th day.



*Mrs. E.*, Age 27. Para 1. Normal, regular cycles. Last menses Sept. 11, 1946:  
 Sept. 26-Oct. 2, 1946—15th-21st days of cycle. Pregnandiol negative  
     Oct. 3, 1946—22nd day of cycle. Pregnandiol positive (x)  
     Oct. 4, 1946—23rd day of cycle. Pregnandiol positive (x)  
     Oct. 5, 1946—24th day of cycle. Pregnandiol negative  
 Oct. 6-Oct. 15, 1946—25th-34th days of cycle. Pregnandiol positive (x)  
     Oct. 16, 1946—35th day of cycle. Pregnandiol positive (xx); Friedman,  
     positive

Two false negative pregnandiol tests were reported on the 41st and 81st days after normal menstruation in patients now known to have been normally pregnant; one false negative Friedman test was obtained on the 32nd day following menstruation.

**Non-pregnant states.** Correct negative pregnandiol tests were reported in 51 of 54 patients in whom pregnancy was subsequently excluded. The Friedman test was correct in all instances. These results are tabulated in Table IV:

TABLE IV. NON-PREGNANT STATES

Cases	Clinical Diagnosis	Pregnandiol Test		Friedman Test	
		Correct negative	False positive	Correct negative	False positive
10	Delayed menses	8	2	10	0
12	Secondary amenorrhea	11	1	12	0
2	Meno-metrorrhagia	2	0	2	0
2	Post-abortion	2	0	2	0
3	Oligomenorrhea	3	0	3	0
1	Ovarian cyst	1	0	1	0
2	Lactation amenorrhea	2	0	2	0
2	Salpingitis-suspected ectopic	2	0	2	0
3	Fibroids	3	0	3	0
1	Ovarian abscess	1	0	1	0
1	Pseudocyesis	1	0	—	—
15	No data	15	0	15	0
—	—	—	—	—	—
54		51	3	53	0

Three false positive pregnandiol tests were reported in patients with menstrual irregularities as follows:

*Mrs. W.S.* Para 0, gravida 0. Age 32. Irregular cycles with prolonged intervals; treated for sterility with equine gonadotropin. Spontaneous menstruation after 48 days of amenorrhea.

*Mrs. T.C.* Delayed menses. No exact data as to menstrual history were available.

*Mrs. K.* Para 2, gravida 2. Age 41. Scant bleeding 9-25-46 to 10-12-46. 11-15-46

Pregnandiol x. Curettage, 11-25-46, showed proliferative endometrium. Normal menses 12-8-46.

In two instances cited, the possibility of delayed ovulation and corpus luteum activity may account for the false positive pregnandiol test. As stated previously, pregnandiol excretion gives consistent evidence of presumptive pregnancy only in the presence of amenorrhea in otherwise regularly menstruating women. False positive pregnancy tests can thus be anticipated when cycles are frequently prolonged and irregular. In such instances the Friedman test should be performed additionally. Understanding of hormonal relationships is as essential for the proper interpretation of pregnancy tests as is knowledge of diseases which influence serologic tests for syphilis. The pregnandiol test, as well as the Friedman test, must be considered aids to the clinical diagnosis, rather than the diagnosis itself.

**Threatened Abortion.** The reports of Browne, Henry and Venning (2) and, more recently, Guterman (6) suggesting the prognostic significance of diminishing or absent pregnandiol excretion in instances of threatened abortion prompted a study of 14 of such cases with our precipitation technic.

(1) *Threatened abortion terminating in actual abortion.* Eight patients with bleeding in early pregnancy were tested repeatedly; positive Friedman tests corroborated the clinical diagnosis of pregnancy in each case. In 7 instances, termination of pregnancy was preceded by one or more negative pregnandiol tests; in 3 cases, strongly positive tests became negative during the period of observation before abortion; in 4 cases, the pregnandiol tests were negative and remained negative despite persistently positive Friedman tests until abortion occurred. One patient (*Mrs. R.B.*) continued to excrete large amounts of pregnandiol despite abortion following spontaneous rupture of the membranes at 5½ months' gestation. The pregnancy was terminated by injections of pituitary extract when abortion appeared inevitable. The fetus was normally developed. It is possible that pregnandiol excretion might have ceased had the abortion been allowed to follow its natural course. It seems likely also that quantitative determinations might have shown a decline in pregnandiol excretion which was not shown by the qualitative precipitation method because of the large amounts normally formed in the second and third trimesters.

The record of one patient of this series is of particular interest.

*Mrs. E.L.* Age 23. Para 0, gravida 1. This patient volunteered to furnish daily samples of urine for pregnandiol studies after a diagnosis of pregnancy was established by Friedman and pregnandiol tests on the 41st day following normal menstruation. With the exception of one day (61st day) positive pregnandiol tests were obtained until the 66th

(2) *Threatened abortion with retention of pregnancy.* Five cases of threatened abortion with spontaneous remission of bleeding and cramps, and one instance of severe uterine cramps without bleeding necessitating hospitalization, were studied in this group. Consistently positive pregnandiol tests were obtained in four cases throughout the duration of symptoms; in one case (*Mrs. H.P.*), negative pregnandiol tests persisting during the bleeding period, later became strongly positive when symptoms subsided. The following cases are worthy of special comment:

*Mrs. F.R.* Age 30. Para 0, gravida 1. A corpus luteum cyst complicating pregnancy was removed on the 84th day after normal menstruation; the remaining ovary contained no corpus luteum. Slight bleeding was noted prior to discharge from the hospital on the 8th post-operative day despite daily doses of 30 mg of progesterone. Intense bleeding and cramps continued during the ensuing 14 days with passage of clots. Pregnan diol tests, with one exception, remained strongly positive. The pregnancy continued uneventfully to normal delivery at term.

*Mrs. H.P.* Age 23. Para 1, gravida 2. Resection of the cystic portion of an ovary containing the corpus luteum was performed on the 46th day after normal menstruation; the corpus luteum was retained. Bleeding began 24 hours after operation and continued for 19 days. Anhydrohydroxy-progesterone (15 mg. daily) was administered orally during this period. Pregnan diol tests remained negative from the 47th until the 75th day when bleeding stopped. Strongly positive tests have been obtained subsequently. The pregnancy is progressing normally.

This small series suggests the practical usefulness of serial pregnandiol determination as an adjuvant to the management of threatened abortion and corroborates the work of Guterman. While there is no evidence to suggest the cause of progesterone deficiency, whether primary corpus luteum failure or disrupted placental function, it seems clear that endocrine factors may be responsible in some instances, as had been previously suspected without proof. It seems equally clear that non-endocrine causes (developmental, mechanical) may be responsible for others, despite normal corpus luteum and/or placental function. The possibility of successful progesterone replacement therapy is suggested in instances where diminishing or absent pregnandiol excretion is detected early in the course of symptoms. The administration of large doses of progesterone (at least 100 mg. daily) would seem essential. At present commercial rates, such amounts are economically prohibitive.

#### SUMMARY

1. The technic of a greatly simplified and rapid test for the detection of urinary pregnandiol is presented.

2. The practical usefulness of this procedure for the diagnosis of pregnancy was demonstrated in 94 pregnant and non-pregnant controls and in 121 unknown urine samples. All known pregnancy control urines gave

positive tests. Negative pregnandiol tests were obtained in all non-pregnant controls except for determinations made during the luteal phase of the normal menstrual cycle. Two false negative reactions were obtained in 67 unknown samples where normal pregnancy was subsequently determined clinically. Three false positive reactions were recorded in 54 unknown samples where pregnancy was later excluded.

3. False positive pregnandiol tests for pregnancy may be obtained during delayed luteal phases of prolonged cycles where late ovulation has occurred. This limits the accuracy of the pregnandiol test which is most reliable in amenorrhea of otherwise normally and regularly menstruating women.

4. The pregnandiol test was applied in serial determinations in 14 instances of threatened abortion. The clinical outcome coincided with laboratory findings in 13 cases. Consistently negative pregnandiol tests foreshadowed abortion in 7 cases; consistently positive pregnandiol excretion or negative tests which became positive coincided with retention of the pregnancy in 6 cases. In one instance of abortion at 5½ months strongly positive qualitative tests were obtained.

5. The high degree of accuracy shown suggests the practical usefulness of the pregnandiol precipitation test as a rapid method for the diagnosis of pregnancy and as an adjuvant to the management of threatened abortion.

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# THE METABOLISM OF SINGLE THERAPEUTIC DOSES OF THE NATURAL ESTROGENS IN HUMAN SUBJECTS\*

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**I**SOLATION and identification studies of the urinary metabolites of massive doses of the natural estrogens in human subjects (1-4) have revealed (a) that estriol apparently is not converted to estrone or estradiol, (b) that estrone and  $\alpha$ -estradiol are partially interconvertible and the injection of either leads to the excretion of estriol also, and (c) that only a small fraction of the injected material is recovered in estrogenically active forms. There is a need (12) for a simple chemical procedure for estimating the extent of destruction, utilization, or interconversion of therapeutic doses of the natural estrogens in human subjects in various states of health and disease.

We have shown that a liquid chromatogram technique may be utilized to fractionate ternary mixtures of crystalline estrone,  $\alpha$ -estradiol, and estriol (9, 10) and that it may be incorporated in a procedure for the fractionation and photometric estimation of the estrogens in human pregnancy urine (10) and in urine with low estrogen titer (11). The following is a report of some preliminary observations on the application of this procedure to a study of the metabolism of therapeutic doses of the natural estrogens in human subjects, as indicated by the photometric estimation of urinary estrogens in the post-injection period.

## EXPERIMENTAL

The individuals from whom the data herein described were derived, were technicians within the institution. They were, therefore, trained in the accurate collection of twenty-four hour urine specimens (8 a.m. to 8 a.m.). No subject presented any menstrual molimina except that associated with the indicated surgery. None was receiving any other form of medication at the time of the tests. The hormone medication† was administered at 5 p.m. and unless otherwise indicated all urine voided after

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† Pharmaceutical preparations were drawn from our pharmacy stock. Parke, Davis & Company preparations of estrone (theelin in oil) and estriol (theelol kapsels), and Schering Corporation preparations of  $\alpha$ -estradiol benzoate (progynon-B) and progesterone (polutin) were used.

treatment on the day of medication was included in the specimen collected the following day. Two hundred ml. of butyl alcohol was used as a preservative for each twenty-four hour specimen. Rest periods of at least one week intervened between each experiment.

### METHOD

Each twenty-four hour urine specimen was extracted with butyl alcohol as previously described (10) and the aqueous extract of the residue therefrom was hydrolyzed as described in a subsequent publication (11). Excretion values for the estrone, estradiol, and estriol fractions were calculated by use of a color correction equation for the Kober reagent (11) following their fractionation by our liquid chromatogram technique (9, 10).

### DISCUSSION

The data from a bilaterally ovariectomized-hysterectomized female following the ingestion of 1.92 mg. of estriol is summarized in Figure 1.

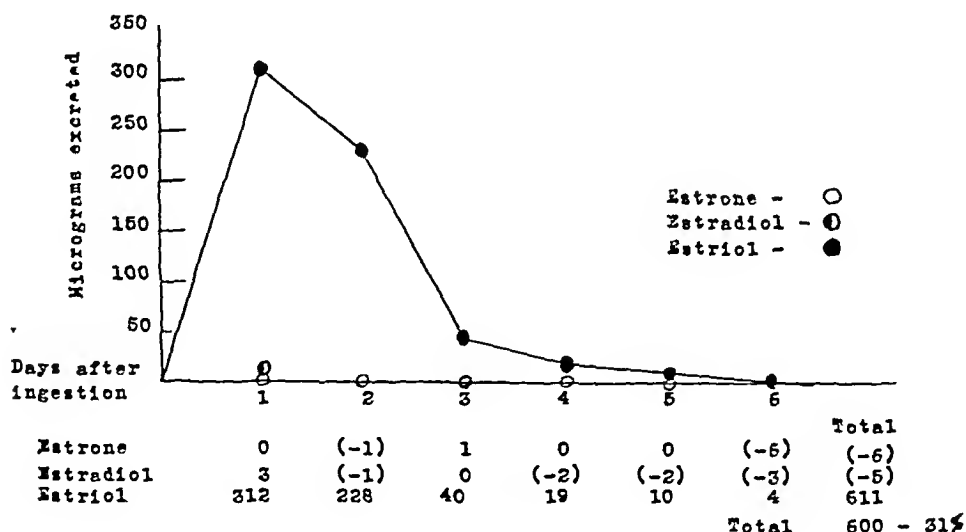


FIG. 1. Photometric estimation of the urinary excretion of estrogens by a bilaterally ovariectomized-hysterectomized human female after the ingestion of 1.92 mg. of estriol. The negative values (in parentheses) arise from the utilization of a color correction equation.

Virtually all of the estrogen excretion occurred during the first three days after ingestion and there is no observable conversion of estriol to estrone and estradiol. The negative excretion values in the table arise from the use of a color correction equation with the Kober reagent (11) which we have shown involves an uncertainty factor of plus-minus 7 micrograms of

estrone, plus-minus 10 micrograms of estradiol, and plus-minus 5 micrograms of estriol per twenty-four hour urine specimen. We have shown (11) that crystalline estrone and  $\alpha$ -estradiol added to our urine extracts immediately after hydrolysis are recovered in the appropriate filtrate fraction in the range of 80 to 100 per cent, and crystalline estriol in the range of 60 to 70 per cent. Therefore the 31 per cent recovery of ingested estriol should represent an actual excretion of at least 40 to 50 per cent. This is in agreement with previously reported work using larger doses of estriol and bioassay techniques (4, 5).

The urinary excretion data following intramuscular injection of 1.0 mg. of estrone in oil into the same human subject is shown in Figure 2. The

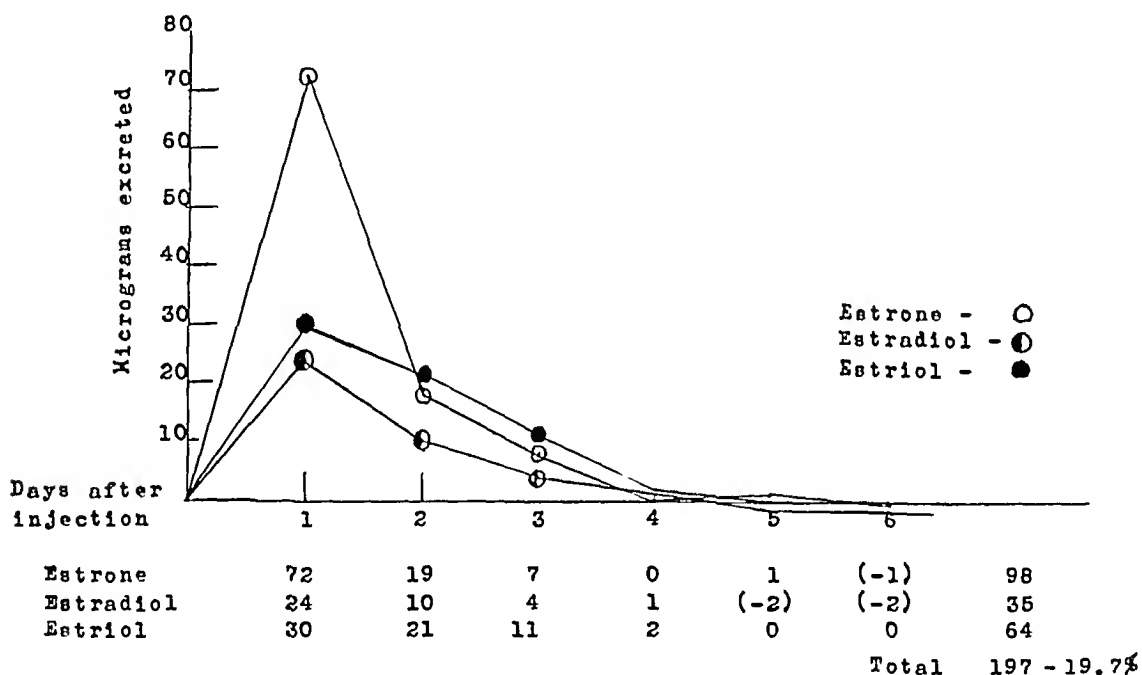


FIG. 2. Photometric estimation of the urinary excretion of estrogens by a bilaterally ovariectomized-hysterectomized human female after the injection of 1.0 mg. of estrone. The negative values (in parentheses) arise from the utilization of a color correction equation.

excretion values of all three fractions for the first twenty-four hours are of sufficient magnitude to indicate conversion of the administered estrone to estradiol and estriol. The total amount of estrogen recovered (19.7 per cent) is significantly less than that recovered from the oral ingestion of the less active estriol (40-50 per cent), but considerably higher than that reported in a similar study on a bioassay basis by Smith et al. (2.8 per cent) (5). Unconverted estrone is the principal estrogenic factor excreted in the first twenty-four hour period; it is displaced thereafter by estriol. This is in

agreement with the findings of Smith et al. (6) for a larger dose of estrone.

The liquid chromatogram technique for the first twenty-four hour urine specimen in the above study was modified so that each chromatographic filtrate fraction totaling 100 ml. of eluent was collected in 20 ml. portions and the estrogen content of each aliquot separately determined. The chromatographic dispersion of the estrogens in these aliquot portions of the eluate is similar to that shown by ternary mixtures of crystalline estrone,  $\alpha$ -estradiol, and estriol in pure solution (9) (Chart 1).

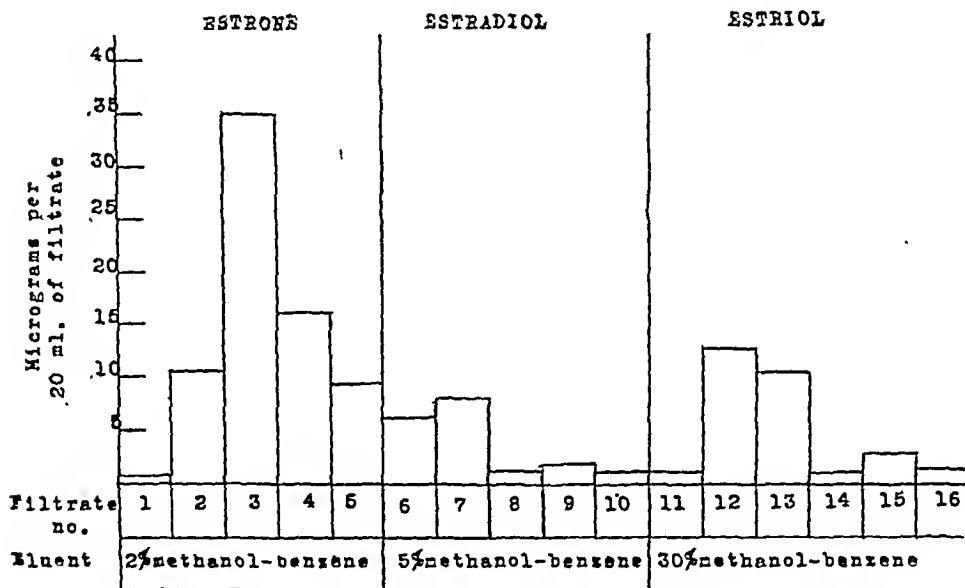


CHART 1. Distribution of urinary estrogens in successive 20 ml. filtrate fractions of a liquid chromatogram prepared from 24-hour urine specimen collected after injection of 1.0 mg. of estrone in a bilaterally ovariectomized-hysterectomized human female.

Schiller and Pineus (4) have shown that massive doses of estrone apparently undergo the same course of estrogen conversion in the human male as has been postulated for the human female. Accordingly, we repeated our study of estrone metabolism in a normal male subject, using 1 mg. of estrone as in the foregoing study. A comparison of these results with those of the first three days of the previous study reveals a remarkable similarity of the two sets of data (Fig. 3). This finding suggests that the absence of ovaries and uterus places the female human subject on approximately the same basis as the normal male, insofar as the capacity to metabolize estrone is concerned.

The urinary excretion data following the injection of 1.6 mg. of  $\alpha$ -



estradiol benzoate equivalent to 1.2 mg. of  $\alpha$ -estradiol into the bilaterally ovariectomized-hysterectomized human female is shown in Figure 4. It is to be noted that the titer for estradiol at no time exceeds the uncertainty factor (10 micrograms) for our procedure. However, there is obvious conversion to estrone and estriol and practically all of the estrogen excretion

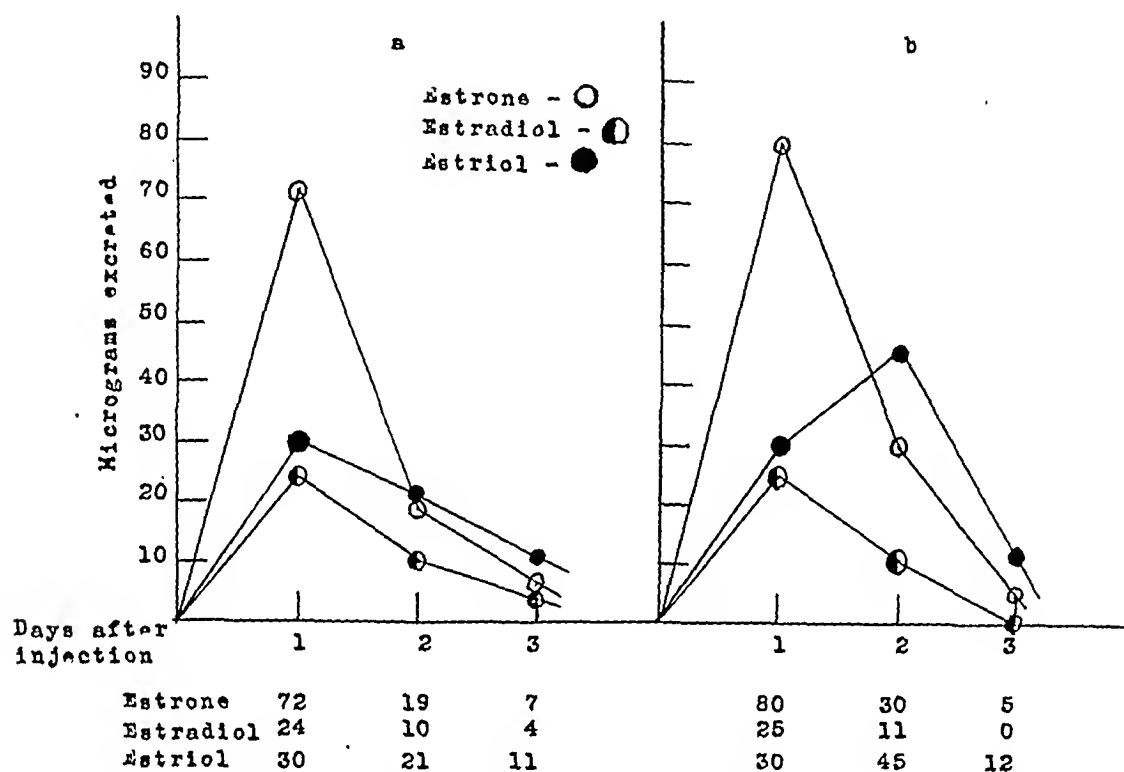


FIG. 3. Comparison of the urinary excretion of estrogens by (a) a bilaterally ovariectomized-hysterectomized human female and (b) a normal human male, after the injection of 1.0 mg. of estrone.

occurs in the first three days in spite of the conjugated state of the injected hormone. The over-all recovery of injected estrogen is significantly less than for either estrone or estriol. A similar study (to be published) following the injection of 2.5 mg. of  $\alpha$ -estradiol dipropionate (equivalent to 1.77 mg. of  $\alpha$ -estradiol) intramuscularly in a castrate male subject revealed practically negligible estrogen excretion. These studies would suggest that conjugation of the female sex hormones before administration does play some role in the manner in which therapeutic doses of the estrogen are metabolized.

An attempt to evaluate the possible role of the intact but unprimed uterus in the metabolism of the natural estrogens was made by repeating some of the previous studies on a bilaterally ovariectomized human female whose uterus was still present. The urinary excretion data following the

single injection of (a) 1.0 mg. of estrone and (b) 1.0 mg. of estrone plus 10.0 mg. of progesterone in such a subject is given in Figure 5. In the absence of progesterone the daily excretion of all three fractions barely exceeded the uncertainty factors for our method. Simultaneous injection of progesterone appears to exert a sparing action on the destruction of in-

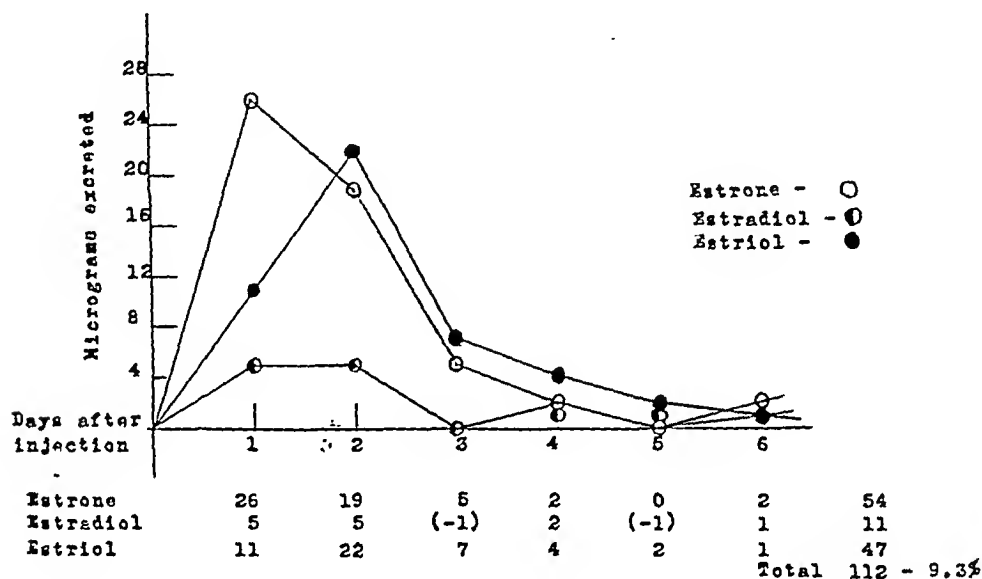


FIG. 4. Photometric estimation of the urinary excretion of estrogens by a bilaterally ovariectomized-hysterectomized human female after the injection of 1.6 mg.  $\alpha$ -estradiol benzoate (equivalent to 1.2 mg.  $\alpha$ -estradiol). The negative values (in parentheses) arise from the utilization of a color correction equation.

jected estrogenic hormone. However, conversion of estrone to estriol is not as remarkable as in the normal male. These studies differ in the time element from those previously described in that the individual was injected at 5 p.m. of the first day of collection. The twenty-four hour collection period was terminated in each instance by the first morning specimen.

In order to determine whether the vehicle in which the estrogen is administered plays a role in the manner in which the estrogen is metabolized we injected 2.0 mg. of estrone in aqueous suspension intramuscularly in the same subject. The data (Fig. 6) on estrogen excretion for the seven-day period including the day of injection indicate that there is little evidence of a peak of excretion which characterizes the other preparations in oil. Presumably there is slower absorption of the hormone from the aqueous media and hence more complete destruction before excretion.

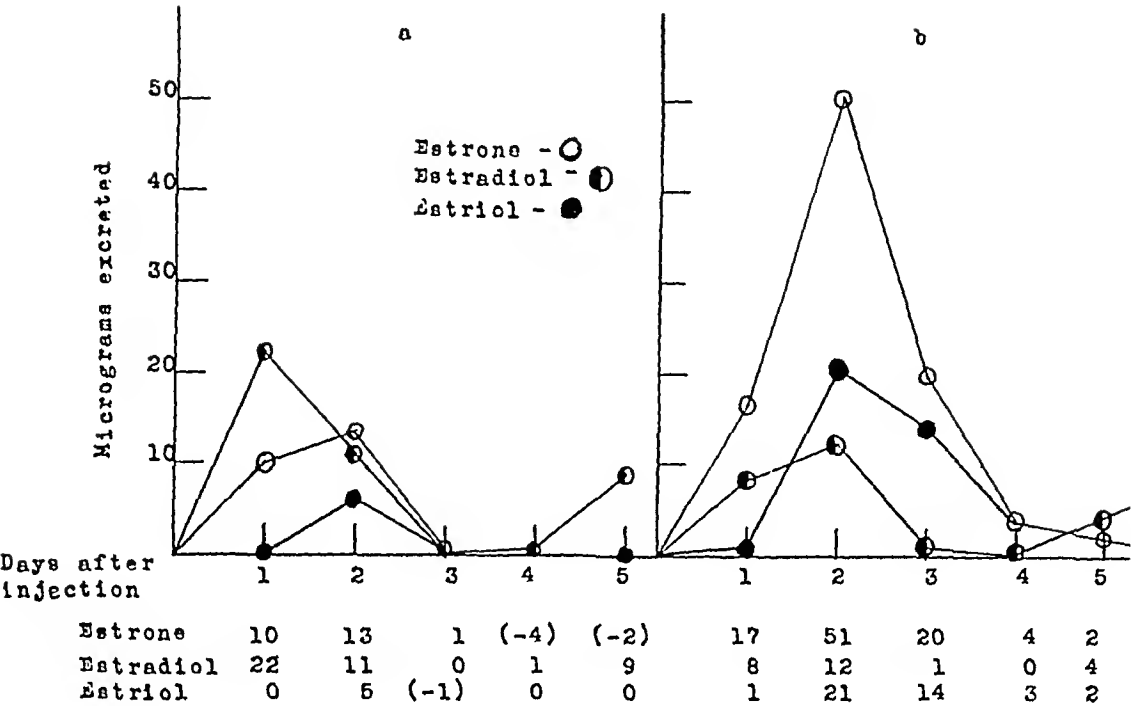


FIG. 5. Photometric estimation of the urinary excretion of estrogens by a bilaterally ovariectomized human female after the injection of (a) 1.0 mg. of estrone, (b) 1.0 mg. of estrone plus 10.0 mg. of progesterone. The negative values (in parentheses) arise from the utilization of a color correction equation.

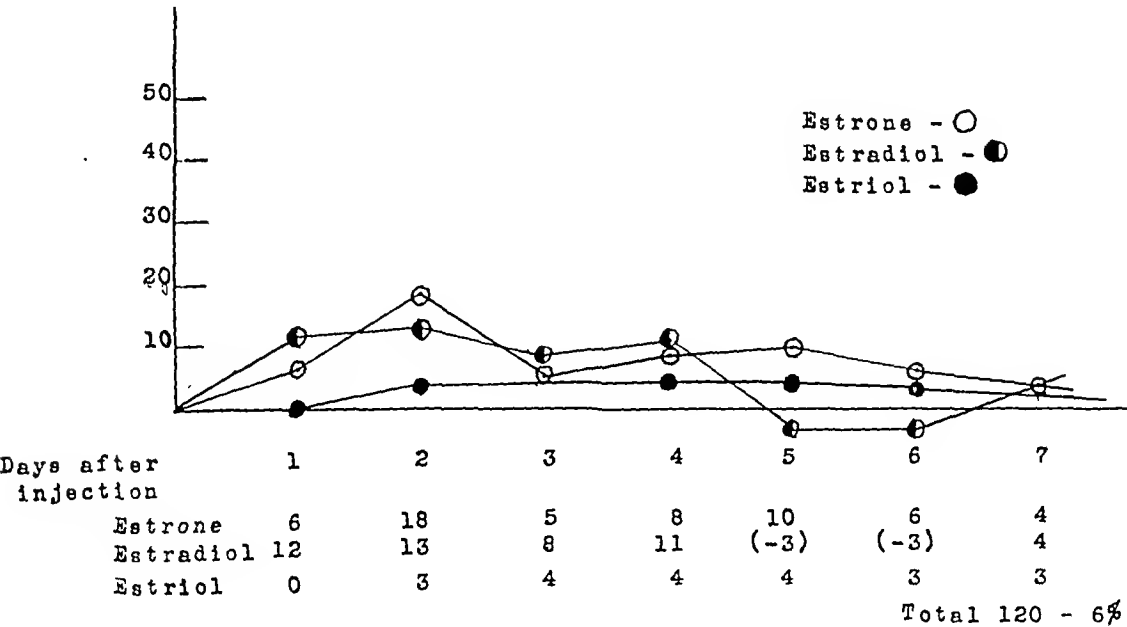


FIG. 6. Photometric estimation of the urinary excretion of estrogens by a bilaterally ovariectomized human female after the injection of 2.0 mg. estrone in aqueous suspension intramuscularly. The negative values (in parentheses) arise from the utilization of a color correction equation.

The urinary estrogen excretion values of a normal human female after the ingestion of 1.44 mg. of estriol (a) during the first half of the menstrual cycle and (b) during the second half of the cycle are summarized in Figure 7. A positive pregnanediol glucuronidate determination was obtained

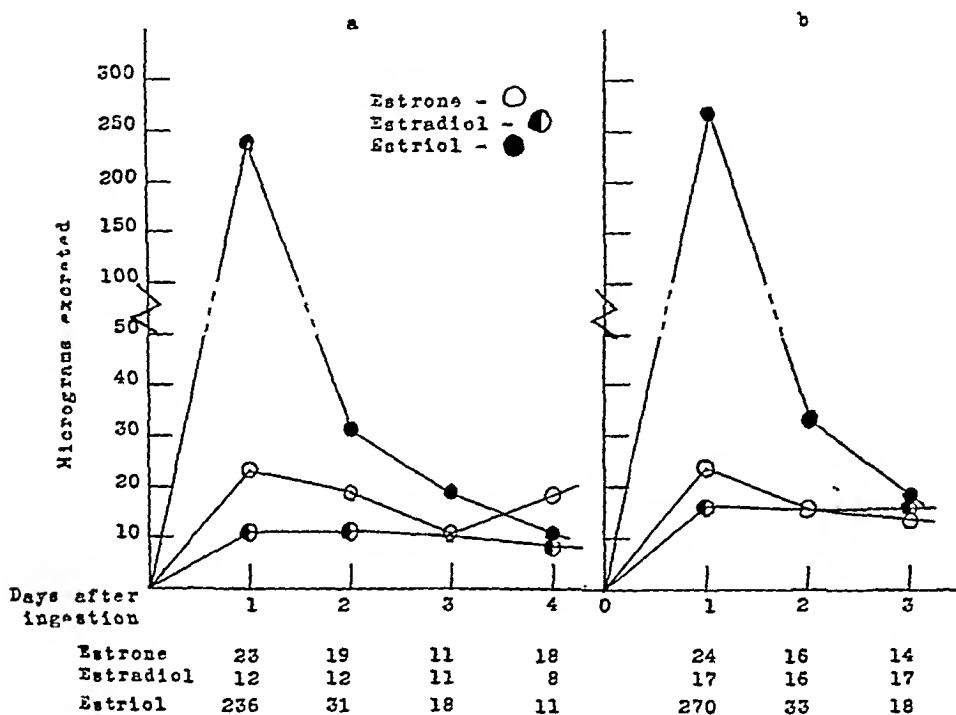


FIG. 7. Urinary excretion of estrogens by a normal human female after ingestion of 1.44 mg. of estriol (a) during the first half of the menstrual cycle, (b) during the second half of the menstrual cycle.

during the second half of the cycle, thus indicating the presence of a progestational endometrium. The excretion of the ingested estriol closely parallels the values obtained following the ingestion of estriol by the bilaterally ovariectomized-hysterectomized female. The amounts of estrogen appearing in the estrone and estradiol fractions are of the same order as those excreted by a normal non-treated female and thus do not represent probable conversion of the estriol.

Urinary estrogen excretion values were determined following the injection of 1.0 mg. of estrone (a) during the first half of the menstrual cycle and (b) during the second half of the cycle, in the case of a normal human female (Fig. 8). In each instance the hormone was injected at 5 p.m. of the first day of collection. The excretion values are not of an order to draw any

clear-cut conclusions. There is indication of a peak of estrogen excretion during the second day but the values closely approximate those associated with non-treated subjects. This elevation was more marked in the second half of the cycle and cannot be attributed to the sparing action of progesterone since our tests for pregnanediol during the second half of this par-

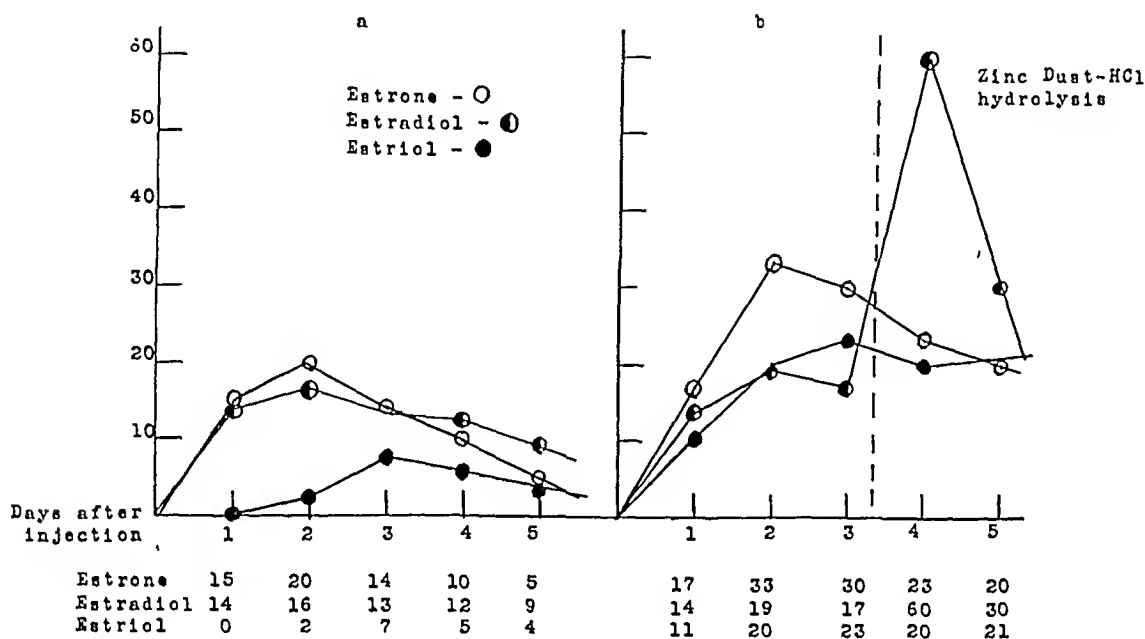


FIG. 8. Photometric estimation of the urinary excretion of estrogens by a normal human female after the injection of (a) 1.0 mg. of estrone during the first half of the menstrual cycle, (b) 1.0 mg. of estrone during the second half of the menstrual cycle. The last two urine specimens were subjected to zinc dust-hydrochloric acid hydrolysis according to the method of Smith and Smith (7).

ticular cycle were negative and it is assumed therefore that a progestational endometrium was not present. The data of days 4 and 5 in the second half of the cycle are worthy of comment since they show the effect of a reducing medium during the hydrolysis of the conjugated urinary estrogens. Smith and Smith (8) have reported that the enhanced estrogenic activity of such urinary extracts is greater than can be accounted for by assuming conversion of estrone to  $\alpha$ -estradiol or more complete hydrolysis. Our limited information on the subject as indicated by the urinary excretion values for days 4 and 5 seems to lend support to their view.

Our data on the metabolism of therapeutic doses of the natural estrogens in human subjects qualitatively agree with the data on massive doses as summarized by Schiller and Pincus (4). Since the work herein reported was of an exploratory nature in order to give some indication of the type of human subjects, of hormone preparations, and of dosages most likely to

yield significant data by our method of fractionation and photometric estimation of urinary estrogens we feel that quantitative comparisons at this time would be premature. Further work of a similar nature is in progress on human subjects with certain types of malignant neoplasms. A report of this work will appear at a later date.

### SUMMARY

Sufficient material has been presented to demonstrate that our method for the fractionation and photometric estimation of the estrogens in human urine is adequate in general to elicit significant information regarding the metabolism of therapeutic doses of the natural estrogens in various stages of health and disease. Preliminary studies indicate that the procedure might possess possibilities in evaluating the functional status of various target organs involved in the metabolism of the natural estrogens.

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## LAURENTIAN HORMONE CONFERENCE

The Laurentian Hormone Conference of the A.A.A.S. will meet from September 8 to September 13, 1947 at the Ste. Adele Lodge, Ste. Adele, Quebec. The following program has been arranged:

### I. *Hormones in Growth and Metabolism*

The Mechanism of Action of the Plant Growth Hormones

Dr. Kenneth V. Thimann, Harvard University

Monday evening, September 8.

The Biochemistry of Pituitary Growth Hormone

Dr. Choh Hao Li, University of California

Tuesday morning, September 9.

The Effects of Hormones, Vitamins and Other Substances on Adrenal Function and Lipid Metabolism

Dr. Robert H. Williams, Thorndike Memorial Laboratory, Boston City Hospital

Tuesday morning, September 9.

### II. *Steroid Hormones*

New Syntheses in the Field of Estrogens

Dr. K. Miescher, Ciba Limited, Basle, Switzerland

Tuesday evening, September 9.

Steroid Excretion in Health and Disease

#### 1. Chemistry

Dr. Seymour Lieberman, Memorial Hospital, New York City

Wednesday morning, September 10.

#### 2. Clinical Aspects

Dr. Konrad Dobriner, Memorial Hospital, New York City

Wednesday morning, September 10.

Urinary Steroids in Adrenal Disease

Dr. H. L. Maxon, Mayo Clinic

Wednesday evening, September 10.

### III. *Histochemical and Histophysical Methods in Hormone Research*

The Chemical Histology of Endocrine Glands

Dr. Edward W. Dempsey, Harvard University

Thursday morning, September 11.

Localization of Radioactive Compounds in Tissues

Dr. C. P. LeBlond, McGill University

Thursday morning, September 11.

### IV. *Testis Physiology and Function*

The Biology of the Interstitial Cells of the Testis

Dr. Charles W. Hooker, Yale University

Thursday evening, September 11.

The Testis in Human Hypogonadism

Dr. Warren O. Nelson, University of Iowa

Friday morning, September 12.

Some Observations as to the Role of Testicular Secretions

Dr. James B. Hamilton, Long Island College of Medicine

Friday morning, September 12.

Regarding the Gonadotrophic Gonad Axis in Men

Dr. Carl G. Heller, University of Oregon

Friday evening, September 12.

V. *Hormones and Hypertension*

The Renal Pressor System and Hypertensive Disease

Dr. A. C. Corcoran, the Cleveland Clinic Foundation

Saturday morning, September 13.

Hypertension as a Disease of Adaption.

Dr. Hans Selye, University of Montreal

Saturday morning, September 13.

Membership in the Conference is limited by the facilities of the Lodge and is therefore established by invitation. Applications for membership by active investigators in the hormone field will be received by the Committee on Arrangements and its advisory committee. The members of the advisory committee are Drs. B. J. Brent, H. M. Evans, H. B. Friedgood, R. G. Hoskins, H. Jensen, F. Koch, I. T. Nathanson, L. T. Samuels, E. Schwenk, M. L. Tainer, A. White. Application for membership may be made up to June 30, 1947.

Conference members are given a special convention rate of \$7.70 (Canadian) a day per person. A limited number of accommodations are available for wives and children of Conference members. Applications should state if reservations for family members are desired.

The Proceedings of the Conference are published as a book by the Academic Press. Volume I is now available under the title *Recent Progress in Hormone Research*.

R. W. BATES

R. D. H. HEARD

GREGORY PINCUS, *Chairman*  
Committee on Arrangements



# Abstracts of

## CURRENT ENDOCRINE LITERATURE

*Editor; D. A. McGINTY. Collaborators: A. R. ABARBANEL, F. N. ANDREWS, B. L. BAKER, F. A. DE LA BALZE, ISRAEL BRAM, R. A. CLEGHORN, RUCKER CLEVELAND, C. D. DAVIS, ANNA FORBES, M. B. GORDON, H. S. GUTERMAN, M. M. HOFFMAN, R. G. HOSKINS, C. D. KOCHAKIAN, H. S. KUPPERMAN, H. L. MASON, JANET W. MCARTHUR, THOMAS H. MCGAVACK, A. E. MEYER, K. E. PASCHIKIS, A. B. PINTO, J. R. REFORZOMEMBRIVES, E. C. REIFENSTEIN, JR., G. G. RUDOLPH, L. T. SAMUELS.*

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### PITUITARY

CRAFTS, R. C. The effects of iron, copper, and thyroxine on the anemia induced by hypophysectomy in the adult female rat. *Am. J. Anat.* 79: 267-291 (1946).

Hypophysectomy of adult female rats induced a microcytic hypochromic anemia accompanied by bone marrow hypoplasia involving the formation of erythrocytes. Since injections of thyroxine, iron and copper prevented the microcytosis and bone marrow hypoplasia, reduced the hypochromia and maintained a normal erythrocyte count, the author concluded that the anemia following hypophysectomy probably results from faulty metabolism which may involve iron.—*B.L.B.*

FELDMAN, F., J. B. ROBERTS, S. SUSSELMAN, AND B. LIPETZ. Coincidence of diabetes mellitus and hypopituitarism. *Arch. Int. Med.* 79: 322 (1947).

This is the case report of a woman 50 years old with diabetes mellitus, in whom the pancreas, thyroid, adrenal cortex and pituitary gland seem to have deviated from normal. At the age of 19, she required a thyroidectomy for hyperthyroidism. At 38, she was found to be diabetic and required insulin in dosages as high as 35 to 50 units daily for a period. Three months before her death, a hypopituitary-hypoadrenal syndrome, including hypoglycemic crises, developed. At autopsy, the pituitary gland was found to be almost completely destroyed, with secondary changes in the adrenal cortices. The patient's diabetes gradually improved as the hypopituitarism became more severe, and she died after a prolonged course in the hospital marked by several hypoglycemic episodes. The relationships of the pituitary, adrenal cortex and pancreas are reviewed with special reference to the so-called Houssay phenomenon. This is apparently the fourth recorded case representing this phenomenon.—*I.B.*

INGRAHAM, F. D., AND H. W. SCOTT, JR. Craniopharyngiomas in children. *J. Pediat.* 29: 95, 1946.

A summary is given of the authors' experience with craniopharyngiomas in 16 children observed at the Children's Hospital and the Peter Bent Brigham Hospital, Boston during 1935 to 1945. Detailed histories of two cases are presented: 1) that of a 6 year

old girl who succumbed to pneumonia after 4 operations, and 2) that of a 13 year old boy with progressive loss of vision and "psychic seizures" who was returned to a normal existence by surgery and roentgen therapy. Embryologic, pathologic, physiologic, and therapeutic aspects of craniopharyngiomas in children are discussed. The pathologic physiology of these 16 cases is described under 4 headings: visual disturbances, intracranial hypertension, hypophyseal disorders, and hypothalamic disorders. The lesions may be located so as to compress either or both the hypophysis and the hypothalamus, with consequent variation in the clinical picture. The most common symptoms in children are headache and vomiting and, in adults, visual disturbances. Progressive loss of vision occurred in 9, arrested growth in 5, and diabetes insipidus in 3 patients. This latter syndrome more commonly developed as a transitory sequel to operation. Fröhlich's syndrome (dystrophia adiposogenitalis) was not encountered. Various overt forms of hypothalamic disorders developed in less than one-third of the cases, usually in the advanced stage after unsuccessful surgery. Ocular and visual field examinations were the most consistent in revealing abnormal physical findings. No laboratory findings were particularly helpful in diagnosis, and indications of anterior pituitary deficiency (depressed basal metabolic rate, reduced blood iodine level, and depressed urinary 17-ketosteroid excretion) were more valuable in following the course of the disease than in establishing the diagnosis. Roentgenograms of the skull showed abnormalities in all of the patients: calcification above the sella turcica was present in 8, calcification in the sella in 2, evidence of increased intracranial pressure (separated sutures, convolutional atrophy, and erosion of clinoid processes) in 7 and enlargement and distortion of the sella turcica in 8. Significant retardation of bone age as estimated from ossification centers of the wrists was encountered in 5 cases. The diagnosis was made without difficulty in 10 of the patients. Surgical exposure of the cyst by transfrontal craniotomy with aspiration of contents and removal of as much as possible of the cyst wall was carried out on all of the cases. Of the series of 16 patients, 11 died from the late effects of the tumor or direct operative complications; and 5 survived, of which two are considered to be "cures." Three children were subjected to 4 craniotomies. The sexes were equally distributed in the groups of patients. Of 190 intracranial tumors verified at the Children's Hospital from 1933 to 1945, 12 were craniopharyngiomas, an incidence of 6.3 per cent in children under 15 years of age. In Cushing's series of 2,000 verified intracranial tumors at all ages, the incidence was 4.6 per cent. The authors conclude that despite the rather discouraging results obtained by surgical treatment, there is no other logical approach to the problem presented by craniopharyngiomas, although a critical appraisal must be made of roentgen therapy as an adjunct to surgery in the management of these tumors.—*E.C.R., Jr.*

## THYROID

FOSS, H. L., AND H. M. KLINGER. Hyperthyroidism without goiter. *Pennsylvania M. J.* 50: 591 (1947).

The use of thiouracil, propylthiouracil and radioactive iodine is considered in detail. The authors point out that hyperthyroidism is encountered even when the thyroid is so small that it fails to constitute a goiter. Hence the condition may not be recognized until after cardiovascular symptoms have developed when cure is difficult or impossible. This inability to demonstrate the existence of a goiter is the chief obstacle to proper diagnosis and early therapy of these cases. The authors favor preoperative preparation of the patient followed by a bilateral thyroid resection.—*I.B.*

GRASSO, R. AND E. DE ROBERTIS. Studies on thyroid stimulating hormone using the cytological method. I. Circulating thyroid stimulating hormone in rats treated with thiourea. *Rev. Soc. argent. de biol.* 22: 79-83 (1946).

The authors have investigated the amount of thyroid stimulating hormone (T.S.H.) in the blood of rats treated with thiourea. 20 animals were treated with thiourea, 1% in the drinking water, for 13 or 18 days. An extract of their blood was injected into the heart of 16 guinea pigs; each guinea pig received an amount of extract corresponding to 2 ml. of blood. 6 control guinea pigs received blood extract of non-treated rats. The thyroids of the guinea pigs were examined 30 minutes after having received the injection and the "cytological coefficient" of these thyroid glands was determined. This "cytological coefficient" is based on the amount of colloid droplets present in the thyroid cells, after desiccation of the glands; according to the authors, it gives an index of the concentration of thyroid stimulating hormone in the blood assayed. With this technique it was found that the blood of the thiourea treated rats, contained an increased concentration of T.S.H. The increase was more marked in the blood of rats treated during 13 days than in those treated for 18 days. The authors interpret their findings as a direct proof that treatment with thiourea produces an increase of circulating T.S.H. in rats.—*J.R.M.*

HERTZ, A., AND A. ROBERTS. Radioactive iodine in the study of thyroid physiology. VII. The use of radioactive iodine therapy in Graves' disease. *West. J. Surg.*, 54: 474-486 (1946).

Cure of thyrotoxicosis in 80% of a series of 29 patients given 5-25 millicuries of radioactive iodine (I 130) followed by ordinary iodine for a period of two to four months is reported.—*J.M.*

HIGGINS, G. M., AND O. R. JONESON. Effect of graded doses of thyroxin on experimental goiters, induced by promizole. *Am. J. M. Sc.* 212: 294 (1946).

Promizole, a sulfone shown to exert a favorable influence on experimental tuberculosis, is likewise goitrogenic. The thyroid hyperplasia observed in animals given promizole could not be prevented by giving large amounts of iodine but was completely controlled by giving thyroxin. Furthermore, the hyperplasia of the thyroid gland did not occur in animals, previously hypophysectomized, when given the goitrogen. This report covers the results of a study undertaken to determine the amount of thyroxin necessary to prevent the changes of the thyroid gland which promizole will induce in animals. The authors summarize their results and conclusions as follows. "1) The administration of d,1-thyroxin, in amounts ranging from 1 to 10  $\mu$ g. daily, to young rats during a period of 28 days, while they were fed a diet containing the goitrogen, promizole, at a level of 0.5%, did not inhibit the usual untoward effects which promizole normally exerts on the growth, appearance and appetite of immature animals. 2) As hitherto shown, promizole depressed thyroid activity. In this experiment the average metabolic rate of animals given promizole in their diet for 28 days was lowered 19.7% from that recorded for the normal control group. Two  $\mu$ g. of d, 1-thyroxin daily increased the average rate 10.4% above that of the control group which received promizole alone, while 4  $\mu$ g. maintained an average oxygen consumption equal to that recorded for the animals which did not

receive either thyroxin or promizole. 3) Promizole exerts a thyroid-stimulating influence through the pituitary gland, which, in this experiment, resulted in the development of goiters weighing more than 3 times the average weight of the thyroid gland in control animals. The administration of 1  $\mu$ g. of d,l-thyroxin daily resulted in the development of goiters which were but slightly less than twice the normal size, while 2  $\mu$ g. daily resulted in maintaining thyroid glands of normal weights. 4) The heights of the thyroid acinar cells were maintained at normal or less than normal levels by giving 2  $\mu$ g. of d,l-thyroxin daily. Without any supplemental thyroxin, the average acinar cell height was more than twice that of the normal gland, while 1  $\mu$ g. of thyroxin was adequate to maintain an average cell height considerably less than twice that of the normal thyroid gland. 5) The changes observed in the colloid of thyroid acini which were induced by promizole were partially prevented by 1  $\mu$ g. of thyroxin per day and were completely nullified by giving 2  $\mu$ g. daily. 6) Promizole appears to exert its goiter stimulating effect by preventing the synthesis of thyroxin by the thyroid cell, thereby permitting an increased elaboration of thyroid stimulating hormone by the anterior lobe. By the administration of small amounts of thyroxin daily during the time the goitrogen was provided, thereby maintaining adequate blood levels of thyroxin, normal thyroid glands were maintained."—*E.C.R., Jr.*

LAHEY, FRANK H. Surgery of the thyroid gland. *New England J. Med.* 236: 46 (1947).

The paper is a review of Dr. Lahey's thirty years experience with the surgery of the thyroid gland. Some of the newer developments and the less-frequent complications of thyroid disease are discussed. After considering the difficulties arising in pre-operative preparation of the patient with thiouracil, a plan using thiouracil followed by iodine is described which obtains the desirable effects of both drugs. Data are presented on the value of thyroidectomy in thyrocardiac disease. The problem of exophthalmos is considered, and an operation for intractable exophthalmos involving orbital decompression is described. The complications of recurrent-nerve paralysis and destruction of parathyroid glands are considered as well as the diagnosis and operative removal of intrathoracic goiters and carcinomas of the thyroid gland.—*L.T.S.*

MOSCHCOWITZ, E. Pathogenesis of cirrhosis of the liver occurring in patients with diffuse toxic goiter. *Arch. Int. Med.* 78: 497 (1946).

This is a useful review of the subject, with a report on a total of 31 patients who died in the Mount Sinai Hospital between the years 1930 and 1944, from diffuse toxic goiter. In 10 of these cases a type of hepatic cirrhosis apparently pathognomonic of diffuse toxic goiter was found. The lesion is identical with that seen in chronic hepatic venous congestion and cardiac cirrhosis, but differs from the latter in that topographically it is not around the central veins but in the interlobar septums, often encroaching on the lobule itself. In the early phases the areas of fibrosis can be traced to the terminal ramifications of the hepatic artery as it passes into the interlobular vascular septums. This artery and its ramifications represent a significant factor of the equalization of intravascular pressures between the hepatic artery and the portal vein within the liver. Apparently the lesion results from the increased velocity of blood flow, an invariable accompaniment of, and almost peculiar to, this disease in the early phases. This altered circulation renders the maintenance of the normal pressure relations between the hepatic artery and the portal vein difficult; eventually decompensation arises, with re-

sulting stasis in these areas, and the lesion begins as capillary congestion. In time, just as in chronic venous congestion of central origin, capillary sclerosis results, with eventual fibrosis. The cirrhosis is predominantly in the subcapsular zone of the liver. As in the ordinary congestive liver, this is due to the resistance offered by the capsule of the liver. The cirrhosis arises only from the smaller subdivisions of the portal spaces, because the interlobular branches of the hepatic artery arise only from such spaces. The cirrhosis bears a definite but not absolute relation to the duration of the malady. Nevertheless, this cirrhosis is sometimes absent in persons who submit a history of apparent long duration of the disease. This may be accounted for by the observation that chronic venous congestion is an exceedingly common sequence of long-standing diffuse toxic goiter, even in patients without cirrhosis, and that this venous congestion neutralizes the increased velocity of blood flow. This contribution should be studied in its entirety to be fully appreciated.—*I B.*

PLANCK, E. H. A comparison of the effectiveness of radiation therapy and estrogenic substances in the management of hyperthyroidism. *South. M. J.* 39(10): 794-799 (1946).

The treatment of hyperthyroidism not associated with clinical enlargement of the thyroid with estrogens and x-ray was presented. Five milligrams of stilbestrol was administered intramuscularly once or twice weekly depending on the severity of the symptoms. 1200-1600 r were administered in six divided doses over a period of three weeks to the patients who received x-ray therapy. A summary of the laboratory findings of 8 patients in each group, before and after treatment, showed that both x-ray and estrogenic therapy caused some improvement as evidenced by decreased thyroid activity. Greater depression of thyroid activity was attained in two patients given combined estrogen and irradiation therapy. Massive doses of estrogens (no dose mentioned) given to an iodine-fast thyrotoxic patient 12 days before surgical intervention reduced the BMR from a plus 82 to a minus 32 and caused a corresponding decrease in pulse rate. No toxic effects or changes in the female sex organs were observed after the administration of estrogens. It is suggested that further observations on the use of estrogenic substances in the treatment of hyperthyroidism should be recorded so that an evaluation of this therapeutic measure could be made.—*H.S.K.*

PUPPEL, I. D., C. P. LEBLOND, ELSIE RILEY, AND G. M. CURTIS. The clinical significance of the functional behavior of adenomas of the thyroid gland. *J. Lab. & Clin. Med.* 31(4): 484-485 (1946).

Radio-iodine fractionation studies of pathologic thyroid tissue removed from seven patients indicated that the cells of all types of nodular or adenomatous thyroid tissue were functionally autonomous. They "consistently showed less avidity for iodine and produced less thyroxine and diiodotyrosine than the groups of thyroid cells in the surrounding thyroid tissue."—*T.J.McG.*

REVENO, W. S. Observations on the use of thiouracil. *Ann. Int. Med.* 25: 822-831 (1946).

From experience derived from the treatment of 70 patients with thiouracil, the author evaluates the drug as follows: (1) Thiouracil is effective in both toxic adenoma and toxic diffuse goiter, exerting its full action after an average of six weeks of administration.

Previous administration of iodine retards the action somewhat. (2) Permanent remission occurs in 18% of cases after stopping the drug. The relapse rate is highest in patients treated less than 18-26 weeks. At present the optimal period of treatment from the standpoint of inducing permanent remission is not known. (3) Toxic reactions occur in 13% of cases. Agranulocytosis has an incidence of 2.5% and a mortality rate of 10 to 12%. (4) Thiouracil is today the most effective agent for inducing remission in thyrotoxicosis and is extremely useful in the preparation of patients for thyroidectomy. Impressions with which many investigators may not agree are: (1) Thiouracil is a valuable treatment for hyperthyroidism induced by overdosage with thyroid substance and for acute thyroiditis. (2) Therapeutic tests with thiouracil are a valuable diagnostic aid in borderline hyperthyroidism.—*J.M.*

SEXTON, D. L. Thiouracil. Clinical evaluation following two and one-half years' experience. *South. M. J.* 39(11): 891-897 (1946).

The author described his experiences with thiouracil in 44 patients, 36 of whom exhibited clinical hyperthyroidism. Thiouracil in doses not exceeding 0.6 grams per day resulted in improvement in 32 of the 36 hyperthyroid patients. Seven of the 44 patients developed reactions, six necessitating discontinuance of the drug. Reactions encountered were leucopenia without agranulocytosis, jaundice, purpura and alopecia. Thiouracil was found to be more advantageous than iodine in preoperative preparation of the hyperthyroid patient since the effect of thiouracil was more lasting and the basal metabolic rate could be depressed below normal. Thiouracil, preoperatively, also reduced the necessity of two-stage operations and the incidence of postoperative thyroid storms. There was no refractoriness to thiouracil therapy. In one of 4 patients with diabetes and hyperthyroidism the diabetes was controlled when the hyperthyroidism was suppressed. Two pregnant women receiving thiouracil were carried to term without any untoward effect on either the mother or the fetus. Although remissions after thiouracil therapy have been reported the author advocated that further cases must be considered before medical treatment of hyperthyroidism with thiouracil can be substituted for the prevalent operative procedures.—*H.S.K.*

WILKINS, L., AND W. FLEISCHMANN. Effects of thyroid on creatine metabolism with a discussion of the mechanism of storage and excretion of creatine bodies. *J. Clin. Investigation* 25(3): 360-377 (1946).

The authors have attempted a critical analysis of our knowledge of the relationship between the thyroid and creatine metabolism, adding observations of their own made in treated and untreated normal, hypogonadal, hyperthyroid and hypothyroid subjects. The following facts were stressed. In relation to the normal, the creatine output is increased in hyperthyroidism, and decreased in thyroid insufficiency; conditions are exactly reversed in the case of creatinine. Therefore creatinine plus creatine remains practically a constant as we pass in any given instance from overfunction to underfunction of the thyroid or vice versa. In rats, muscle creatine and phosphocreatine are decreased in hyperthyroidism and increased in hypothyroidism. In thyrotoxic patients treated with iodine or thiouracil, the rise in creatinine excretion is not as consistent as the fall in creatine output. The effects of the thyroid treatment of hypothyroidism fall into two phases. In the first of these, lasting about 30 days, total creatine plus total creatinine rises above the hypothyroid level. In the second period, the total excretion of these two substances is the same as prior to treatment, but the creatine excretion is increased with a con-

comitant decrease in the creatinine. This increase in creatine varies to some extent with the amount of thyroid given. The increase in creatine is attributed by the authors to a loss of muscle creatine, and the decrease in creatinine to a decrease in the conversion of creatine to creatinine. Testosterone propionate causes a decrease in the excretion of creatine and creatinine, probably due to the fact that the body needs increased amounts of creatine under such steroid stimulus. The increased creatine and creatinine excretion caused by methyltestosterone is believed to be due to the fact that this preparation in addition to increasing the need of the body for creatine also materially increases its formation. Following the withdrawal of therapy with either testosterone or methyl testosterone, there is a temporary increase in the release of creatine in both instances. Thyroid deficiency does not prevent, or in any other way apparently interfere, with the formation and increased output of creatine produced by methyl testosterone. However, when methyl testosterone is discontinued in patients with hypothyroidism, instead of a temporary increase in the excretion of creatine there is an immediate sometimes rather rapid drop in such excretion to the pretreatment level. This is apparently due to the fact that the hypothyroid patient retains creatine more tenaciously than the normal individual. In the light of all the experimental and clinical data, the authors discuss the synthesis of creatine, physiological factors such as age and nutritional state that influence its formation and excretion, and other questions in relation to thyroid and gonadal status as they affect and are influenced by creatine metabolism. In contrast to the lability of creatine excretion, are the relatively small changes in creatinine output in any given individual under a wide variety of conditions. In the opinion of the authors, "the creatinine output depends upon the total stores of creatine in the muscle which might be subject to variation rather than upon the muscle mass," for in many instances, the change is greater than can be accounted for on the latter currently accepted theory. —*T.H. McG.*

WOLMAN, I. J. Basal metabolism in childhood: current progress. *Am. J. M. Sc.* 211: 733 (1946).

The author gives a critical review of the major contributions on the more controversial aspects of the subject, and the interpretations offered, as expressed in the medical literature in the past 5 years. The subjects discussed include: definition, methods, observations (sleep, is preliminary training of the subject necessary?, infants, children of pre-school age, childhood period, adolescence, diagnosis of hypothyroidism, and influence of growth). He summarizes the review as follows. "Many tables for the resting oxygen consumption of normal children have been gathered to serve for the evaluation of the status of other individual children. These standards are expressed customarily in terms of units of age, or height, or weight, or surface area, or of several of these growth measures in combination. Age is the least satisfactory as a direct basis of reference. There is no universal agreement as to whether height, or weight, or surface area, with or without reference to age, work out the best in actual practice. With children of average or normal body build any of the different types of standards seems to work well. But when the body build is atypical or distorted, as, for example, with obese children or dwarfs, the different standards give results which fail to conform one with another. The normal range for individual variation is greater in childhood than in adult life. One reason for this appears to be the growth impulse, which stimulates the metabolism to an as yet unmeasured degree. Obviously, we must work out more fully the importance of growth, and of the thyroid gland, and of other controlling factors now only guessed at. Until then

the physiologic significance of basal metabolism measurements in childhood must remain obscure and of little clinical value in the presence of prominent deviations from the patterns of most normal children."—*E.C.R., Jr.*

## PARATHYROID

BAKER, B. L., AND J. H. LEEK. The relationship of the parathyroid glands to the action of estrogen on bone. *Am. J. Physiol.* 147(3):522-526 (1946).

Fifty-two female rats of the Long-Evans strain, ranging in age from 32-90 days at the start of the experiment, were studied. Alpha-estradiol dipropionate in peanut oil was injected subcutaneously on alternate days for 20 days at the rate of 250 micrograms per injection in intact and parathyroidectomized rats. Studies of the estrogen-treated animals showed that "hyperossification" could be induced in the absence of the parathyroid glands. The authors concluded that even though the hypophysis is essential to this phenomenon, the parathyroids are unnecessary and, therefore, the secretion of a parathyrotropic factor by the pituitary need not be postulated.—*F.N.A.*

NORRIS, E. H. Anatomical evidence of prenatal function of the human parathyroid glands. *Anat. Rec.* 96: 129-142 (1946).

From a study of the parathyroids of 139 embryos, fetuses and newborns, the author concluded that the parathyroids of the fetus function during intra-uterine life. This conclusion was based on the occurrence of five cell types in the fetal parathyroid differentiated on the basis of size and density of the cytoplasm, on the similarity of these cells to those of post-natal life, the individuality of the growth rate of the fetal parathyroid gland, and on the early histological differentiation of this organ. No details were given of the techniques which were employed.—*B.L.B.*

ROGERS, H. M., F. R. KEATING, JR., C. G. MORLOCK, AND W. W. BARKER. Primary hypertrophy and hyperplasia of the parathyroid glands associated with duodenal ulcer. *Arch. Int. Med.*: 79: 307 (1947).

In this patient, pronounced clear cell hyperplasia of the parathyroid glands and primary hyperparathyroidism were associated with healed duodenal ulcer. The patient was a white male aged 68 years, first seen at the Mayo Clinic in 1919 when posterior gastroenterostomy was performed because of duodenal ulcer. Death on October 5, 1945, resulted from renal insufficiency secondary to nephrocalcinosis. Peripheral gangrene had developed as a terminal event. The parathyroid glands weighed 47.56 Gm. Pancreatic lithiasis and mild osteitis fibrosa cystica were also disclosed at necropsy. An unusual type of necrosis of arterial walls, with intimal calcification, appeared responsible for gangrene of the peripheral parts. Gastrointestinal symptoms, which were presumed during life to be due entirely to ulcer, appeared in retrospect to have been manifestations of severe hyperparathyroidism.—*I.B.*

## ADRENAL

JAUDON, J. C. Addison's disease in children—(critical review). *J. Pediat.* 28: 737 (1946).

The author has collected reports of 100 cases of Addison's disease in children under



15 years of age. He lists 62 cases as proven, 23 as probable and 15 as doubtful. A case classified as proven satisfied the following criteria: "evidence either of characteristic blood chemical changes, obvious improvement following adequate therapy over a long period, or autopsy findings demonstrating inadequacy of adrenal cortical tissue." Of these 62 proven cases the etiology was tuberculosis in 53 (86.9 per cent.) with bilateral involvement in 50, atrophy of both glands without evidence of tuberculosis in 5 (8 per cent.), and adrenal cortical hyperplasia with macrogenitosomia in 3 (4.8 per cent.). Six cases were still alive at the time of the report, and in two of these Addison's disease was thought to result from tuberculosis, in two others from adrenal cortical atrophy, and in the others from adrenal cortical hyperplasia. The age at the onset of symptoms was under 2 years in 3 (4.9 per cent.), from 2 to 5 years in 3 (4.9 per cent.), from 5 to 10 years in 7 (11.5 per cent.), and from 10 to 15 years in 48 (78.7 per cent.). The youngest child on record developed symptoms at 8 days of age and was diagnosed at 3 weeks of age; this patient is still alive and doing well on desoxycorticosterone in propylene glycol (3 drops five to six times a day sublingually). The first case below 15 years of age was reported by Ogle in 1856, seven years after Addison's original description. It is concluded that Addison's disease should be considered to be a clinical and not a pathological entity which includes any process which directly or indirectly destroys the adrenal cortex sufficiently to produce the signs and symptoms described by Addison. The diagnosis is rendered difficult in some cases by the presence of hyperplasia of the androgenic zone of the adrenal cortex. Therapy in children with Addison's disease is mentioned briefly.—*E.C.R., Jr.*

## OVARIES

BOURNE, ALECK. Endocrines in gynecology. *Brit. M. J.*, 1: 79 (1947).

The author disusses the difficulties in the clinical use of hormones associated with the female gonadal cycle. Emphasis is laid on the need for more carefully controlled clinical research and the need for better assay methods for blood and urine. Quantitative studies on the relations between emotional state and secretion of the pituitary hormone as well as the relation of nutrition to endocrine activity were suggested.—*L.T.S.*

FARRIS, E. J. The time of ovulation in the monkey. *Anat. Rec.* 95: 337-345 (1946).

The injection of post-ovulatory urine of women and monkeys into immature rats has been found to cause a hyperemia of the ovaries. In seven monkeys in which the test was positive for five successive days, ovulation was confirmed by laparotomy; in four monkeys in which the test was negative, absence of ovulation was demonstrated by laparotomy or bimanual examination.—*B.L.B.*

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## SYNDROME OF RUDIMENTARY OVARIES WITH ESTROGENIC INSUFFICIENCY AND INCREASE IN GONADOTROPINS

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FOR MANY years it seemed reasonable, when a patient presented retardation of growth and lack of genital development, to accept these two phenomena as being due to a primary hypophyseal lesion, provided that cretinism, severe disorders of nutrition and other conditions with general impairment could be excluded.

The knowledge gained concerning the relations between the anterior pituitary gland and the gonads, and particularly the investigations of the hormones of these glands in the urine, have made possible a better understanding and explanation of several of these syndromes. Worthy of mention in this respect are the initial discoveries made by Zondek, and Smith and Engle, showing definitely the hypophyseal-gonadal relations. Subsequently as a result of these investigations, biological tests have appeared which allowed the identification and measure of certain hypophyseal hormones and illustrated many concepts of the physiopathology of these conditions. Based mainly on the application of these biological tests, two groups of American investigators, Varney and his collaborators (1942) (33) and Albright and his co-workers (1942) (1) established the clinical and hormonal characteristics of a new syndrome and published various observations on this subject. The former authors called it "Association of short stature

retarded sexual development and high urinary gonadotropin titers in women," the latter "A syndrome characterized by primary ovarian insufficiency and decreased stature. A report of 11 cases with a digression on hormonal control of axillary and pubic hair." Later, more cases, with ovarian biopsies, were presented by Wilkins and Fleischmann (1944) (34-35). These investigators called this condition "ovarian agenesis."

The observation of various patients, whose clinical histories will be presented in this paper, has allowed us to prove that their symptoms and signs parallel the characteristics of this new syndrome.

The biopsies obtained from six of these patients showed only the existence of rudimentary ovarian structures, and in none of the histological preparations was it possible to demonstrate differentiated elements characterizing the ovary. In all of the patients, the sexual characteristics showed evidence of estrogenic insufficiency, and repeated determinations of the gonadotropins in the urine gave titers higher than the normal range.

Since this syndrome has an anatomical basis it is highly improbable that surgeons and pathologists have overlooked it. Thus, before 1942, there appeared in the literature isolated observations on cases which undoubtedly corresponded to the syndrome of rudimentary ovary, since they showed considerable alterations in the sexual organs as proved by laparotomies and necropsies.

Nevertheless, these publications certainly do not diminish the merits of the work of Varney, Albright and their respective co-workers, since these investigators were the first to explain these cases by means of biological tests, indispensable in our opinion, to verify the diagnosis. These authors have classified with a clinical approach a group of patients, suffering from obvious endocrine disturbances, and set up the basis for their treatment. It may be added that this new clinical picture has helped to understand and apply to human beings a great part of the knowledge gained in experimental endocrine physiology.

The observations previous to 1942 which, we might say, correspond to the anatomical period of the syndrome, may be divided into two groups:

- a) Those gained at necropsies.
- b) Those obtained at laparotomies.

Table 1 contains a summary of the bibliographic indications together with the diagnostic elements of both groups. It concerns twenty observations that appeared between 1912 and 1941, thirteen of which correspond to the necropsy group and seven to the group of biopsies.

This series of cases belongs to the period during which each author reported the result of his experience on a merely anatomical basis. It was not until later, as has already been mentioned, that publications appeared which no longer dealt with scattered cases but with series of cases which

TABLE 1

Observation No.	Author and year	Necropsy or biopsy	Age	Height in cm.	Menstruations	Mammary glands	Hair		Ovaries	
							Pubic	Axillary	Macroscopic examination	Histological examination
1	Kermanner, P. (12) (1912)	Biopsy	24	short	0	+	+	0	Filiform formations, 3 X 5 cm long	—
2	Ravon (22) (1913)	Biopsy	27	135	0	0	0	0	Have not been found	—
3	Clivet (17) (1925)	Necropsy	38	145	0	0	+	+	Have not been found	—
4	Schulze (25) (1925)	Necropsy	30	—	—	0	+	+	Have not been found	—
5	Sulheim (28) (1921)	Biopsy	21	101	0	0	+	0	2.5 X 7.5 mm in size	Primordial follicles and interstitial cells
6	Handerath (21) (1926)	Necropsy	61	145	0	0	+	+	Only a thin whitish fold	Ovarian theca has not been encountered
7	Schürmann (20) (1927)	Necropsy	25	143	0	0	0	0	Have not been found	—
8	Baur, W. (2) (1927)	Biopsy	23	45	0	0	+	0	—	Lack of differentiated elements; cells similar to those of the glomerular zone of the suboral cortex
9	Herscheimer (10) (1929)	Necropsy	—	—	—	—	—	—	Have not been found	—
10	Riesle and Wal-lert (21) (1930)	Necropsy	30	123½	0	0	0	0	Filiform formations	Lack of differentiated elements, Nodules of the adrenal cortex of Marchand and sympathic cells
11	Kolzig (14) (1930)	Biopsy	25	174	0	0	+	+	Have not been found	—
12	Meyer, R. (16) (1931)	Biopsy	26	169	0	0	+	+	Filiform formations	Lack of differentiated elements
13	Prusol (26) (1931)	Necropsy	45	137	0	0	±	0	Have not been found Tuberculous peritonitis	Absence of differentiated elements
14	Goldwasser (6) (1933)	Biopsy	23	141	0	0	0	0	Filiform formations	Lack of differentiated elements
15	Pela (18) (1935)	Necropsy	34	185	0	0	±	0	Formations of the size of the seeds of oranges	Germinal epithelium. Lack of primordial follicles
16	Trouet (31) (1938)	Necropsy	20	153	0	0	±	0	Formations 0 X 2.5 mm large	Germinal epithelium. Lack of differentiated elements
17	Pich (10) (1938)	Necropsy	18	137	0	0	±	0	Filiform formations	Lack of differentiated elements
18	Pich (10) (1938)	Necropsy	40	155	0	0	±	0	Filiform formations	Interstitial cells, Primordial follicles have not been found
19	Pich (10) (1938)	Necropsy	20	163	0	0	0	0	Right ovary 28 X 3 mm, Left ovary 30 X 1 X 2 mm.	Germinal epithelium and some primordial follicles
20	Sharpey-Schafer (20) (1911)	Necropsy	21	145	0	0	±	0	Have not been found Tuberculosis of peritoneum	—

may be included in the new syndrome for anatomical, physiopathological and clinical reasons.

Review of the literature and careful study of the results of the observations permit us to describe the anatomical, biological and clinical manifestation of the "Rudimentary Ovary" syndrome, as follows:

- a) The patients are generally of short stature of moderate degree.
- b) Primary amenorrhea is present.
- c) The breasts have not developed, the external genitalia have an infantile appearance, and the uterus is hypoplastic.
- d) The pubic and axillary hair is almost always diminished and sometimes absent.
- e) The anatomical study of the ovary shows its rudimentary condition.
- f) The hypophyseal gonadotropins are always increased in the urine.
- g) The estrogens in the urine show subnormal titers.
- h) The neutral 17-ketosteroids of the urine are diminished.
- i) The vaginal smears are of the atrophic type.
- j) There are the following alterations in the skeletal system:
  - 1) delay in aging of bone,
  - 2) diffuse osteoporosis,
  - 3) vertebral chondrodystrophia.
- k) Congenital anomalies have been mentioned in certain cases: webbing of the neck, cubitus valgus, syndactylism, coarctation of the aorta, etc.

In order to establish the clinical diagnosis of this syndrome, it is necessary to find the following combination of symptoms: primary amenorrhea, increase in urinary hypophyseal gonadotropins and the presence of the other manifestations produced by estrogenic insufficiency.

The diagnosis becomes indubitable when the histological study discloses the existence of rudimentary ovaries. Yet we must admit that this histological finding is not essential and that it is possible to reach the same result by means of the clinical elements and the biological tests mentioned above.

#### MATERIAL AND METHOD

The hypophyseal follicle-stimulating-hormone (F.S.H.) in the urine was measured by Zondek's method with the modifications introduced by Albright and his co-workers (1943) (13). In order to determine the titers of the F.S.H., one of its biological properties was used, the power to increase the weight of the uterus of the prepuberal female mouse following estrogen liberation from its own ovary. With this method the F.S.H. titers were established corresponding to those of normal women as well as the increases and decreases observed in different conditions. It has been settled that normal adult women have more than 6 mouse units and less than 96 mouse units in the urine per 24 hours.

The urinary estrogens were measured according to D'Amour and Gustavson's method (1930) (7). It is considered that the urine excreted in 24 hours by normal adult women contains from 30 to 160 I.U.

For the determination of the neutral 17-ketosteroids in the urine Zimmermann's method (1936) (36) was employed with the modifications introduced by Callow and his co-workers (1939) (4). The values indicated correspond always to the urine per 24 hours. Twelve to eighteen mg. are considered to be normal values for the adult woman in this period of time.

In order to perform the insulin tolerance test, the instructions given by Reifstein (1944) (23) were followed. One tenth unit of regular insulin per kilogram of ideal weight was administered and samples of the blood were taken while fasting and 20, 30, 45, 60, 90 and 120 minutes after the injection.

The clinical histories of eight patients are presented; only the interesting features and the biological findings considered to be of value for the characterization of the syndrome under discussion are reported.

*Case 1.*—*T.R.*, 19 years of age, was transferred to us by a physician because of lack of menstruation and insufficient development of her secondary sexual characteristics. The physical growth had been normal, and it had never been noted that she was short, as compared with other girls of the same age. When she was 14, scanty pubic hair appeared but no axillary hair was noted. The breasts remained infantile. At that time the patient's personality altered, she became irritable; abnormal and involuntary movements of the muscles of the face appeared, and she had some difficulty in picking up objects. These disorders, which at the beginning had been noticed only once a day and which were of short duration, increased in intensity and occurred several times a day, obliging her to stay in bed for three months (Sydenham's chorea?). Treated with injections, the composition of which she does not know, she improved gradually until the trouble disappeared almost completely.

Born at full term, she had been breast fed by her mother. She began talking at the usual age. She did not remember being ill during her childhood. At the age of 7, she fell from a 3-meter height and was unconscious for several hours. She entered school at the age of 6, and went on for two years, showing some difficulty in retaining what she had learned.

She did not suffer from physical or psychical asthenia, nor from impairment of vision. At the age of 18, she was treated with injectable medicines and after a few months' treatment, a slight and painless bloody discharge from the vagina was observed which lasted only two days.

Her father, a chronic alcoholic, suffers from pulmonary T.B.C. She has 7 brothers and 7 sisters, all of them apparently healthy. The sisters show normal development.

**PHYSICAL EXAMINATION:** Height 166 cm., span 166 cm.; weight 55 kg. Well nourished girl.

**Pilous system:** Lack of axillary hair; mons veneris: hair very scanty, forming a thin stripe.

**Bone system:** Cubitus valgus on the left. Increased pronation of the feet. Partial syndactylism of two toes of the right foot.

Mammary glands: Lack of glandular tissue; very scanty fatty tissue; infantile nipples and areolae (Fig. 1).

Circulatory system: Normal; blood pressure 110/70.

Genitalia: Hypoplastic vulva; labia minora and clitoris small. Hymen preserved. Perineum excavated. Vaginal discharge of an alkaline reaction. Rectal examination: long vagina at the end of which a small stump the size of a thimble is felt, corresponding to a very slightly developed uterus. The ovaries are not felt.

Roentgenograms revealed accentuated carpal osteoporosis; normal spine; small sella

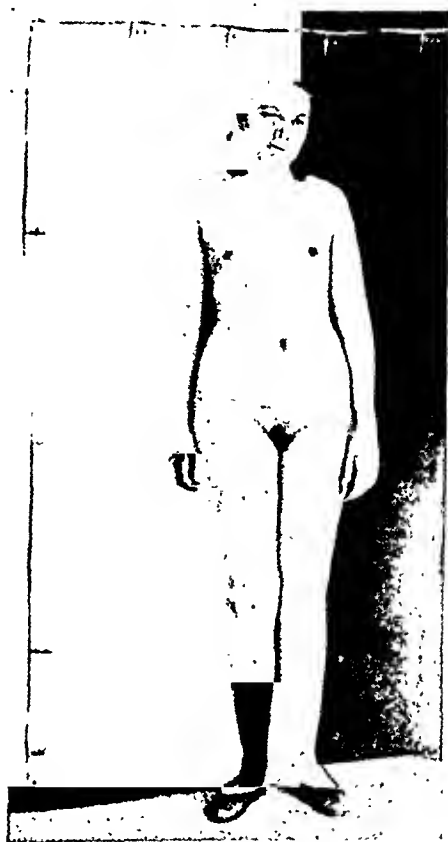


FIG. 1. *Case 1.*—*T. R.*, 19 years of age, height 166 cm.

turcica; epiphyses of the cubitus, metacarpal bones and knees not united, i.e., persistent conjugation cartilages (Fig. 2 & Fig. 3).

LABORATORY DATA: Hypophyseal follicle-stimulating-hormone (F.S.H.)

Nov. 10, 1945, positive for 96 mouse units in the urine per 24 hours.

Nov. 27, 1945, positive for 96 mouse units in the urine per 24 hours.

Nov. 30, 1945, positive for 96 mouse units in the urine per 24 hours.

Aschheim-Zondek test in rats: negative.

17-ketosteroids: Nov. 1945: 7.2 and 12 mg. in the urine per 24 hours.

17-ketosteroids and alarm-reaction for surgical intervention:

Before the operation: 7.2 mg.

After the operation: first day, 14.9 mg.; second day, 12 mg.; fourth day, 5.9 mg.

17-ketosteroids and administration of chorionic gonadotropins:

Before: 12 mg.

After receiving injections of 1,000 rat units per day for five days: 15.2 mg.

Blood cell count: normal.—Blood urea: 32 mg. per cent.

Cholesterol: 131 mg. per cent; 200 mg. per cent; 156 mg. per cent (in three successive blood tests).

Wassermann and Kahn: negative.



FIG. 2. Case 1.—*T. R.*, 19 years of age. Persistence of the conjugation cartilages in the epiphyses of the cubitus and radius, in the metacarpal bones and phalanges. Delay in bone age and osteoporosis of the carpus bones.

Urine: Density: 1.025; acid reaction. Absence of pathological elements.

Renal concentration test: Highest density: 1.032

Robinson, Power and Kepler test: negative.

Carbohydrate metabolism: Exton-Rose test: fasting 64 mg. per cent; after 30 min. 95 mg. per cent; after 60 min. 103 mg. per cent.

Insulin tolerance curve: fasting 99 mg. per cent; 20 min. 52 mg. per cent; 30 min.



53 mg. per cent; 45 min. 88 mg. per cent; 60 min. 99 mg. per cent; 90 min. 105 mg. per cent; 120 min. 99 mg. per cent.

Vaginal smears: Atrophic.

Ocular Fundus and Visual Field: normal.



FIG. 3. The same patient as in Fig. 2. Persistence of the conjugation cartilages and osteoporosis.

VAGINOSCOPY: pink mucous membrane with whitish secretion. Cervix with a smooth mucous membrane, external orifice very small and round.

SURGICAL INTERVENTION: The patient underwent operation for chronic appendicitis, during which it was noted that the uterus was composed of a thickening of muscle tissue, the size of the fleshy tip of the fore-finger; the tubes were thin and increased in length; the ovaries consisted of two whitish cords, the same calibre as the tubes. A right ovariectomy was undertaken.

**HISTOLOGICAL EXAMINATION:** Specific elements of the ovary are not found; only in the cortical region a stroma is noted, ovarian in appearance, but fibrous, such as may be observed on the senile involutive ovaries. A few small ducts are seen to have a cuboidal epithelium, which has nothing to do with the elements peculiar to the ovary and which corresponds perhaps to celomic inclusions (Fig. 4).

**HISTOLOGICAL DIAGNOSIS:** Rudimentary ovary.

*Summary of Case 1.*—Normal growth, bone age retarded, primary amenorrhea, infantile mammary glands, pubic hair very scanty, axillary hair had never developed, hypoplastic vulva, infantile uterus, vaginal smears without estrogenic response, 17-



FIG. 4. Section of ovarian tissue (*Case No. 1, T. R.*). Specific elements are not observed. In the cortical zone stroma of ovarian appearance of the senile involutive type.

ketosteroids diminished, urinary gonadotropins (F.S.H.) increased on several determinations. Syndactylism of the toes, cubitus valgus. The biopsy disclosed absence of the characteristic elements of the ovary.

*Case 2.*—*L.T.R.*, 18 years of age, was first seen in November, 1945, because of lack of sexual development and retardation of growth.

During the school age, she had always been one of the smallest girls, and though she grew later on, her body height had always been shorter than that of her school friends of the same age. Her parents and uncles are of rather tall stature.

Only daughter; born at full term; fed with artificial lactation; began walking at the age of 20 months and talking with some delay. She had measles at the age of 10, and epidemic parotitis when she was 9. At the age of 14, she noted the appearance of thin

pubic and axillary hair, but very scanty. At the same time, a slight increase in the size of the breasts was noted. Until the present time there have been neither menstrual cycles nor bloody genital discharges. She does not complain of "hot flashes"; she has neither visual disorders nor headaches. She is not nervous and her psyche is normal, though rather infantile. She does not suffer from physical or psychic asthenia. She had received no treatment before she came to the hospital.

**PHYSICAL EXAMINATION:** Height 145 cm.; span 152 cm.; weight 40 kg. She is fairly well nourished. Her facial appearance is that of a much older person (Fig. 5).



FIG. 5. Case 2, patient L. T. R. Shows the accentuated cubitus valgus, just as described by Turner.

**Pilous system:** On the skin of the shoulder and arms the hair is abundant. The axillae show scanty hair; a small amount of pubic hair with feminine distribution is noted.

**Skeleton:** Cubitus valgus, more accentuated on the right side; scoliosis and slight vertebral lordosis; the 6th and 7th dorsal vertebrae are depressed and painful on pressure. Pes cavus at rest. Left foot in marked pronation. Articular lassitude.

**Mammary glands:** Lack of glandular tissue, very little fatty tissue, areolae and nipples infantile.

**Genitalia:** Vulva and labia majora hypotrophic with scanty hair reaching the peri-

neum. Abundant vaginal discharge, labia minora deficiently developed, except for the portion near the prepuce of the clitoris. Clitoris of a larger size than normal. Hymen preserved. A small nodule corresponding to a very hypoplastic uterus is felt per rectum.

Vaginosecopy: (done with the urethroscope): Pink mucous membrane, small cervix with a smooth mucous membrane, adolescent type.

Roentgenograms revealed accentuated chondrodystrophia of the dorsal vertebrae, epiphyses not united with the vertebrae; sella turcica normal; the skull with the diploes very thin, and non fusion of epiphyses in the wrists and shoulders. Vertebral and iliac osteoporosis. Osseous development: less than 18 years (Fig. 6-7).



FIG. 6. Case No. 2.—L. T. R., 18 years of age. Delay in bone age.

LABORATORY DATA: Hypophyseal follicle-stimulating-hormone (F.S.H.)

Nov. 27, 1945, positive for 96 mouse units in the urine per 24 hours.

March 12, 1946, positive for 96 mouse units in the urine per 24 hours.

Aschheim-Zondek test: negative.

17-ketosteroids: April 17, 1946, 24.1 mg.; second sample 28 mg., May 8, 1946, 16.3 mg.; May 13, 1946, 16.9 mg.

Urinary estrogens: negative for 24 i.u. (2 rat units) in the urine per 24 hours.

Blood cell count: normal.—Blood cholesterol: 153 mg. per cent.

Blood urea: 25 mg. per cent.—Wassermann and Kahn Tests: negative.

Urinary creatine<sup>1</sup>: 219 mg. per 24 hours.

<sup>1</sup> The creatine and creatinine values refer in all patients to 24 hour samples.

Creatinine: 89 mg. Creatinine Index: 23.2.

Carbohydrate metabolism: Extton-Rose test: fasting 116 mg. per cent; after 30 min. 120 mg. per cent; after 60 min. 147 mg. per cent.

Insulin tolerance curve: fasting 98 mg. per cent; after 20 min. 54; after 30 min. 46; after 45 min. 60; after 60 min. 83; after 90 min. 89; after 120 min. 72 mg. per cent.



FIG. 7. The same patient as in Fig. 6. Accentuated chondrodystrophia of the dorsal vertebrae. Vertebral epiphyses not united.

Basal metabolic rate: +41 and +28 per cent in two determinations.

Urine: Alkaline reaction. Density: 1.012. Abundant urobilin and microorganisms.

Test for highest renal concentration: density 1.027.

Robinson, Power and Kepler Test: negative.

Vaginal smears: atrophic.

OCULAR FUNDUS: normal. Campimetry: narrowing of the visual fields.

SURGICAL INTERVENTION: Laparotomy with Pfannenstiel's incision. Broad ligaments very thin; very small uterus (4-5 cm. long). At the sites corresponding to the ovaries two

cords of whitish color are noted,  $\frac{1}{2}$  em. in diameter. The right adnexa is removed for biopsy.

**HISTOLOGY:** medullary (Dr. Colillas): What should be the ovary is a rudimentary cortex and a medullary zone very rich in vessels. On the border of a section of the tube, a small conglomerate of cells is noted, possibly an inclusion of aberrant cells of the adrenal cortex. Tube hypoplastic. Müllerian and Wolffian residues and a nerve rim surrounded by a cover of sympathetic cells are seen. Lack of differentiated elements of the ovarian parenchyma (Fig. 8).

**HISTOLOGICAL DIAGNOSIS:** Rudimentary ovary. Adrenal inclusions.



FIG. 8. Biopsy of Case 2 (L. T.-R.). Cell conglomerate of the aberrant adrenal cell type. Differentiated elements of the ovary are not noted.

**Summary of Case 2.**—Growth moderately diminished; proportions of the skeleton similar to those in eunuchoidism; bone age delayed; primary amenorrhea. Mammary glands infantile; pubic and axillary hair diminished; vulva hypoplastic; uterus infantile; vaginal smears atrophic; urinary gonadotropins increased above normal. 17-ketosteroids increased (patient suffers from a discrete hirsutism); the insulin tolerance curve shows the reaction to hypoglycemia somewhat diminished; creatine in urine increased. Cubitus valgus; concentric narrowing of the visual fields. Rudimentary ovaries corroborated by the study of the biopsy.

**Case 3.**—R. L., 20 years of age, was first seen in December, 1945, on account of her short stature and because she had never had menstrual periods nor genital bloody dis-

charges. She informed us that until the age of 14, she was steadily growing, but that she had always been one of the smallest among the pupils of her school-year. At fourteen she had stopped growing. When she was 15, scanty pubic and axillary hair appeared, without development of the mammary glands until the age of 19. At that age a slight increase in size had been noted. Since October, 1945, she felt occasional "hot flashes." Sometimes headaches, no visual disorders.

Born at full term, she began talking at the usual age, but walking was delayed. From the second to the ninth year she suffered from diarrheal crises with several daily evacuations, which disappeared at the age of 10. During her infancy, measles and roseola. She went to school until she was 12; at that time she finished the sixth grade, having been a rather bad pupil. She has always been strong, without feeling physical or psychical fatigue.



FIG. 9. *Case 3.—R. L.* Note the pyknic habitus and the lipodystrophy.

The parents are healthy, short in stature. A brother, aged 22, is normal and of average height.

For five months during the past five years she had been treated with thyroid preparations and thereafter with 30 injections of hypophyseal extract. Two years ago, an hypophyseal implantation had been performed. These treatments did not cause any appreciable improvement.

PHYSICAL EXAMINATION: Height 135 cm.; span 140 cm.; weight 46 kg. Fairly well nourished. Strength preserved (Fig. 9).

Pilous system: Discrete hypertrichosis on the forearms. Lack of axillary hair; the pubic hair is considerably diminished.

Mammary glands: There is no development of glandular tissue, the nipples are infantile, with abundant fatty tissue.

Blood pressure (Baumanometer) 138/78. Distribution of the fatty tissue particularly on the girdle area and anterior wall of the abdomen.

Skeleton: Pes cavus corrected simply by standing. Feet show bilateral pronation, more pronounced on the left side. Articular lassitude increased in the superior and inferior members.

Genitalia: Vulva hypoplastic, hymen preserved, some alkaline discharge. A small stump is noted by rectum which seems to correspond to a very hypoplastic uterus.

Vaginoscopy: Pink vaginal mucous membrane; very small cervix.

Anterior and lateral roentgenograms of the spine showed discrete chondrodystrophia of the dorsal vertebrae. Persistence of the conjugation cartilages of the phalanges and inferior extremity of the cubitus and radius; sella turcica normal. Very discrete osteoporosis. Bone age: less than 19 years.

LABORATORY DATA: Hypophyseal follicle-stimulating-hormone (F.S.H.):

Nov. 18, 1945, positive for 96 mouse units in the urine per 24 hours.

Dec. 18, 1945, positive for 96 mouse units in the urine per 24 hours.

April 4, 1945, positive for 96 mouse units in the urine per 24 hours.

Aschheim-Zondek Test: negative.

17-kestosteroids: Dec. 1945: 9 mg. in the urine, per 24 hours; May 8, 1946: 8 mg. in the urine, per 24 hours.

17-ketosteroids and alarm-reaction for surgical intervention:

Before the operation: 8.9 mg.

After the operation: first day 9.2 mg.; second day 14.6 mg.; third day 13.9 mg.; fourth day 5.5 mg.

Urinary estrogens: negative for 24 i.v. in the urine per 24 hours.

Blood cell count: normal.—Cholesterol: 153 mg. per cent.

Urea: 27 mg. per cent.—Renal concentration test: 1.032.

Creatine in urine: 400 mg. per 24 hours.

Creatine + creatinine: 560 mg. per 24 hours.

Creatinine coefficient: 11.4.

Wassermann and Kahn: negative.—Urine examination: density 1.026 normal.

Carbohydrate metabolism: Exton-Rose test: in fasting 98 mg. per cent; after 30 min. 115 mg. per cent; after 60 min. 120 mg. per cent.

Insulin tolerance curve: Fasting 94 mg. per cent; after 20 min. 53; after 30 min. 71; after 45 min. 56; after 60 min. 58; after 90 min. 74; after 120 min. 62 mg. per cent.

Vaginal smears: Atrophic on several examinations.

OCULAR FUNDUS: normal. Visual fields concentrically narrowed.

SURGICAL INTERVENTION: Pfannenstiel laparotomy; broad ligaments very thin, nearly transparent. The uterus has the form of an hour-glass, 4-4½ cm. long; the round ligaments are scarcely visible; the tubes are large but very thin with very small pavilions. Beneath the pavilions some whitish cords are noted, ½ cm. thick, corresponding to the ovaries. The right adnexa is removed. An appendectomy is performed.

HISTOLOGICAL EXAMINATION: (Dr. Colillas): Some colonic cysts are observed within a fibrous stroma. In these cysts the theca layer is not visible. In several zones specific



stroma, somewhat fibrosed, are observed. Ovarian follicles are not noticed. Tubes of essentially normal appearance and congestion of their walls.

**HISTOLOGICAL DIAGNOSIS:** Rudimentary ovary.

*Summary of Case 3.*—Accentuated decrease in stature with proportions of the skeleton similar to those of eunuchoidism; primary amenorrhea; lack of mammary glands; scanty pubic and axillary hair; hypoplastic vulva; infantile uterus; atrophic vaginal smears; urinary gonadotropins increased on repeated determinations; 17-ketosteroids diminished; vertebral chondrodystrophia and diffuse osteoporosis; creatine in the urine increased; creatinine coefficient diminished; the insulin tolerance curve shows that the reaction to hypoglycemia is also diminished; there are no congenital malformations; ocular fundus normal; concentric narrowing of the visual fields. The histological study discloses the absence of the characteristic elements of the ovary.

*Case 4.*—R. V., 14 years of age, came to our Service having noticed that she had stopped growing during the last 3 years and that her sexual development was abnormal.

Since childhood, her stature has been shorter than that of her companions of the same age, and she occupied always the first place among the girls of her school-year. She passed regularly through the primary school and followed as a good pupil the superior course of the Commercial School. At the age of 13, pubic and axillary hair of scanty quantity appeared. Never has she had menstruations; her mammary glands developed very little. A year ago hot flashes appeared in an irregular manner, two or three times a day.

Born normally at full term, walking and talking occurred at the normal age. At the age of 16, she had diphtheria with subsequent paralysis of the soft palate. Her father is healthy, 180 cm. tall; her mother, 162 cm.

The patient was treated in 1944 with hypophyseal extract, 1 cc. three times a week for 2½ months. At the same time, estrogen containing pomades were applied to the breasts. An increase in their volume and pigmentation of the nipples were noted with this treatment.

In April, 1945, she was treated with hypophyseal and adrenal extracts for one month. She believes that in the last year she has grown two centimeters.

**PHYSICAL EXAMINATION:** Height 140 cm.; span 142 cm.; weight 47.2 kg. Fairly well nourished. Facial appearance according to her age. Slight obesity of the anterior wall of the abdomen (Fig. 10).

**Pilous system:** The axillary hair is very scanty. The pubic hair is actually developed as a result of the administration of estrogens.

**Skeleton:** Very exaggerated bilateral cubitus valgus.

**Mammary glands:** in both breasts glandular tissue of the size of a nut is felt (which has appeared after the local application of estrogen-containing pomades). Nipples and areolae very pigmented.

**Circulatory system;** Arterial pressure 125/78 (Baumanometer).

**Genitalia:** External genitalia hypoplastic, more accentuated on the labia minora; clitoris with normal prepuce. There is no discharge; alkaline vaginal secretion; hymen preserved. No uterus is felt per rectum, and on its anatomical site only a small cord is encountered.

Roentgenography revealed generalized osteoporosis but no chondrodystrophia. The rest of the ilium was not united. Bone age corresponded to that of an 18 year old.

**LABORATORY DATA:** Hypophyseal follicle-stimulating-hormone (F.S.H.):

Nov., 1945, positive for 96 mouse units in the urine per 24 hours.

Dec., 1945, positive for 96 mouse units in the urine per 24 hours.

17-ketosteroids: 6 mg. in the urine per 24 hours.—Another determination: 6.5 mg. in the urine per 24 hours.

Postoperative alarm-reaction and 17-ketosteroids:

Before the operation: 5.9 mg.

After the operation: first day, 16.2 mg.; second day, 18.8 mg.; third day, 16 mg.; fourth day, 9.4 mg.; fifth day, 4.5 mg.

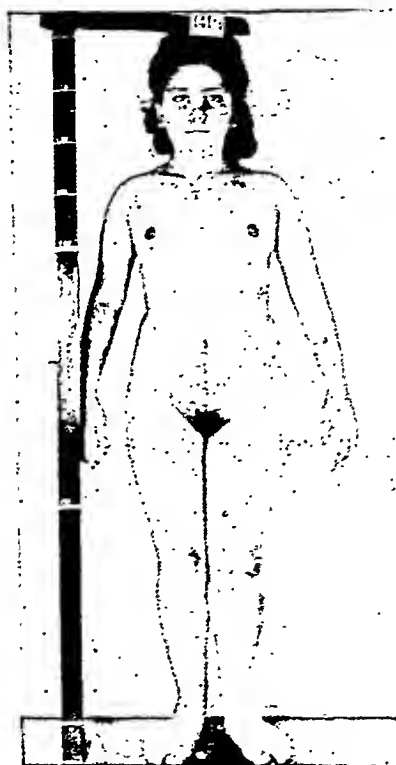


FIG. 10. Case 4, patient R. Y. Height 141½ cm.

17-ketosteroids and administration of chorionic gonadotropins;

Before the administration: 6.5 mg. per 24 hours.

After the injection of 1,000 rat units of chorionic gonadotropins each day for 5 days: 17.1 mg.

Blood cell count: red cells 4,010,000; white cells 9,800; hemoglobin 74%.

Wassermann and Kahn reactions: negative.

Carbohydrate metabolism: Fasting glycemia: 103 mg. per cent.

Vaginal smears: Atrophic.

**SURGICAL INTERVENTION:** A laparotomy was performed disclosing a small uterus, 3

cm. long and 2 cm. wide; rudimentary round ligaments; very small tubes and pavilions; at the site corresponding to the ovaries, two very thin whitish cords were noted. A piece from the left side was removed.

**HISTOLOGICAL EXAMINATION:** (Dr. Colillas): The appearance of the stroma resembles, particularly in certain zones, that of the ovary. A series of inclusions of the epithelial or mesothelial type were observed; some adopted pseudoglandular and canalicular arrangements, others presented themselves as massive islands of cells of variable type. Some of them were interpreted as not belonging to the ovary itself (Müllerian and Wolffian ducts, etc.), and the others as specific of the ovarian pattern but disturbed in their evolution.



FIG. 11. *Case 4 (R. V.).* Stroma similar to that of the ovary. Inclusions of epithelial or mesothelial type, adopting pseudo-glandular arrangements.

Several small foci were seen or noted with the characters of the specific stroma of the ovary, from which seem to generate cells with clear cytoplasm (thecal cells) (Fig. 11).

**HISTOLOGICAL DIAGNOSIS:** Rudimentary ovary.

*Summary of Case 4.*—Growth diminished since her infancy; bone age until the present time normal; primary amenorrhea; mammary glands moderately developed as a result of estrogenic treatment; pubic and axillary hair scanty; vulva hypoplastic; vaginal smears atrophic; 17-ketosteroids diminished; urinary gonadotropins (F.S.H.) increased on repeated determinations; cubitus valgus. Microscopically, the characteristic elements of the ovary are absent.

*Case 5.*—*S. F.*, 22 years of age, was first seen in March, 1946, because of her short stature and lack of sexual development. The body growth continued, though slowly, until

the age of twelve and since then, she had stopped growing almost completely. She had been treated with growth-promoting hormones administered intermittently and claims to have grown several centimeters.

Until 1942, at the age of 18, there was no bloody discharge from the vagina and after that year, coinciding always with the application of injections (anterior hypophyseal lobe), she had the following episodes of bleeding: in 1942, 1943 and 1944, two little bloody genital discharges each year; in February, 1945, only several drops of blood. At the age of 15, pubic and axillary hair appeared in a small quantity. Never had she had hot flashes. Vision was normal, but she suffered from headaches of moderate intensity. She tired easily. She went to school for 3 years and was a rather bad pupil. The patient was born at full term and fed by her mother. During her early infancy she suffered in her country (Russia) from severe rickets which retarded walking till she was 3½ years old.



FIG. 12. Case 5, patient S. F., 22 years of age. Senile facies.

Also dentition had been considerably retarded. She had measles at the age of 6; chronic otitis media persisted until the age of 13. She has always been considered a weak child. During the last years she suffered from diarrheal crises.

The father died at the age of 51 due to trauma; he was a tall man. Her mother was small, though taller than the patient.

**PHYSICAL EXAMINATION:** Stature 136 cm.; span 142 cm.; weight 48 kg. Fairly well nourished. Her facial appearance is that of a much older woman (see photograph) with periorbital wrinkles and wrinkles on the commissure of the lips (Fig. 12).

**Pilous system:** Thin, abundant hair. A small amount of hair on the eye-brows. Scanty axillary and pubic hair.

**Skeleton;** Articular cracks in the right knee. Proportions similar to those in eunuchoidism. Dorsal outward bending of the spine. Slight right cubitus valgus, incomplete

extension of the right elbow. Foot in slight pronation with depression of the arch.

Circulatory system: Normal; arterial pressure (Baumanometer) 120/70.

Genitalia: Vulva: labia majora hypoplastic; labia minora prominent in their superior portions, prepuce and clitoris well developed. Hymen preserved. Vaginotomy; vaginal mucous membrane red; cervix small. Rectal examination: a small hard uterus (4-6 cm.) is palpated; ligaments are not.

ROENTGEN RAYS: anterior and lateral views of spine, discrete chondrodystrophia. Epiphyses of the cubitus and radius not completely united, nor the crest of the ilium. Diffuse osteoporosis. Bone age less than 20 years.

LABORATORY DATA: Hypophyseal follicle-stimulating-hormone (F.S.H.).

Dec. 4, 1945, positive for 96 mouse units in the urine per 24 hours.

Dec. 5, 1945, positive for 96 mouse units in the urine per 24 hours.

March 16, 1946, positive for 96 mouse units in the urine per 24 hours.

March 18, 1946, positive for 96 mouse units in the urine per 24 hours.

Aschheim and Zondek Reaction: negative.

17-ketosteroids: April 17, 1946, 11 mg. per 24 hr.; May 8, 1946, 11.3 mg. per 24 hr.

Carbohydrate metabolism: Exton-Rose test: fasting 86 mg. per cent; after 30 min.

95 mg. per cent; after 60 min. 105 mg. per cent.

Insulin tolerance curve: fasting 82 mg. per cent; after 20 min. 56; after 30 min. 47;

after 45 min. 68; after 60 min. 79; after 90 min. 79; after 120 min. 90 mg. per cent.

Blood cell count: Normal.—90 mg. per cent. Urea: 39 mg. per cent.

Cholesterol: 142 and 181 mg. per cent.—Wassermann and Kahn: negative.

Urine: normal.—Test for highest renal concentration: density: 1.032.

Creatine: in the urine per 24 hours 0.764 Gm.

Creatine + creatinine: 1.960 Gm.—Creatinine: 1.196 Gm.

Creatinine coefficient: 30.7.—Basal metabolism: +25 per cent.

Vaginal smears: atrophic.

Robinson, Power and Kepler Test: negative.

OCULAR FUNDUS AND VISUAL FIELD: normal.

*Summary of Case 5.*—Marked decrease in stature since her childhood; proportions similar to those of eunuchoidism; primary amenorrhea; lack of development of the mammary glands; small amount of pubic and axillary hair; bone age retarded; vulva hypoplastic; uterus infantile; vaginal smears atrophic; urinary gonadotropins increased (F.S.H.); 17-ketosteroids somewhat diminished; diffuse osteoporosis and marked chondrodystrophia of the spine; creatine increased; creatinine index normal.

*Case 6.*—*M. J. M.*, 17 years of age, came to see us on account of her short stature and because up to the present time she had never had menstrual periods, (Fig. 13).

Since the third year of age, it has been noted that she was undersized as compared to other girls of her age. The patient has shown us a series of photographs taken at successive dates, on which she is accompanied by other girls of similar ages; these photographs indicate that the growth has been steady, though retarded (Fig. 14). She had always been looked upon as a weak child; when she was 8, she weighed 18 kgs. At the age of 6, she had difficulty entering school on account of her small height. Until the present time, no bloody discharges from the vagina have appeared; there is no pubic or axillary hair. In 1945, she had several crises with palpitations, suffocations, hot flashes with reddening of the face; oppression in the chest and general lassitude, ending usually in a flood of

tears. These crises lasted from one to two hours and obliged her to go to bed. She tires easily, is irritable and is of easy emotivity.

Normally born at full term, she was fed by her mother for three months and thereafter by a nurse till she was 6 months old. She began talking at the age of one year; dentition and walking occurred at the normal age. She entered school when she was 8 and attended classes for six years as a good pupil. She suffered from gastro-intestinal disorders during early infancy. She had scarlet fever at 6, and measles at 9. When she was 10, she underwent a tonsillectomy and appendectomy and suffered from pneumonia.

Her father is healthy and tall, her mother's height is 155 cm. She has two sisters; one, 18 years of age, is 160 cm. tall and the other, 15 years, is 165 cm.

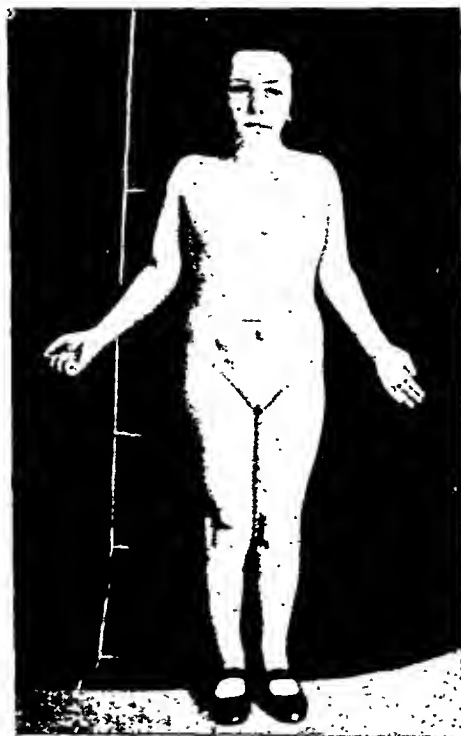


FIG. 13a. Case 6. M. J. M., 17 years of age.

She has been treated with chorionic and blood serum gonadotropins and with thyroid preparations, which have been well tolerated. During these treatments she has had only once a scanty genital blood loss, lasting one day; yet it did not occur a second time in spite of the continued medication.

**PHYSICAL EXAMINATION:** Stature 131 cm.; span 127 cm.; weight 35 kg. Well nourished; white skin with slightly yellowish spots on the face. Hairy hyperkeratosis of limbs. Hair without special characteristics. Lack of axillary and pubic hair. Webbing of the neck, which we would prefer to designate as "Sphynx neck."

**Skeleton:** Bilateral cubitus valgus, more pronounced on the left side. Pain on pressure

in the region of the third and fourth dorsal vertebrae. Slight outside rotation of the feet.

Mammary glands: Not developed; nipples and areolae infantile.

Circulatory system: First sound reduplicated at the apex; systolic slight murmur at the apex; systolic murmur accentuated at the left interscapulo vertebral space. Absence of other signs of aortic coarctation. Blood pressure 130/80 (Baumanometer).

Genitalia: Marked hypoplasia of the external genitalia; hymen preserved. Only a small uterine stump was made out by rectal examination.

Vaginoscopy: pink vaginal mucous membrane; cervix infantile has a wrinkled mucous membrane.



FIG. 13 b.

FIG. 13 c.

*Case 6. MJM.* Same patient as in 13a at the ages of 9 and 13 years, compared with her younger sister.

**ROENTGEN RAYS:** Anterior and lateral films of the spine: vertebral chondrodystrophic lesions. Epiphyses of cubitus, radius and phalanges not closed. Osteoporosis of the spine; cuneiform vertebrae. Skull: thin diploe; small sella turcica. Bone age less than 18 years.

**LABORATORY DATA:** The hypophyseal follicle-stimulating-hormone (F.S.H.).  
Nov. 16, 1945, positive for 96 mouse units in the urine per 24 hours.  
Dec. 22, 1945, positive for 96 mouse units in the urine per 24 hours.  
March 12, 1946, positive for 96 mouse units in the urine per 24 hours.

Ashheim-Zondek Test: negative.

17-ketosteroids: April 17: 19.0 mg.; April 20, 6 mg.; April 27, 8.6 mg.; May 8, 10.5 mg.; May 13, 10.8 mg.

Blood cell count: normal.—Blood Urea: 30 and 40 mg. per cent.

Cholesterol: 238 and 250 mg. per cent.—Inorganic phosphorus: 4.70 mg. per cent.

Blood calcium: 10.6 mg. per cent.—Wassermann and Kahn: negative.

Urine: normal. Density 1.026.

Creatine: 69 mg. in the urine per 24 hours.

Creatine+creatinine: 339 mg. in the urine per 24 hours.

Creatinine coefficient: 97.

Glucose tolerance test: Exton-Rose Test: fasting 78 mg. per cent; after 30 min. 97; after 60 min. 1.04 mg. per cent.

Insulin tolerance curve: Fasting 86 mg. per cent; after 20 min. 43; after 30 min. 54; after 45 min. 50; after 60 min. 58; after 90 min. 58; after 120 min. 62 mg. per cent.

Vaginal smears: atrophic.

Examination of the feces: neutral fats and acid fats not observed. Several digested muscle fibers; absence of blood and parasites.

Basal metabolic rate: +21 per cent.

OCULAR FUNDUS AND VISUAL FIELD: Narrowing of the visual fields. Fundus normal



Fig. 14. Three patients (*Cases 1, 2 and 7*). The patient in the center (*Case 1*) with normal stature, and the two others with relatively reduced growth.

*Summary of Case 6.*—Very marked decrease in growth since infancy; primary amenorrhea; lack of pubic and axillary hair; lack of mammary glands; hypoplastic external genitalia; very small uterus; atrophic vaginal smears; increased urinary gonadotropins (F.S.H.); 17-ketosteroids diminished; diffuse osteoporosis and chondrodystrophia; creatine in the urine increased; creatinine index very increased; insulin curve hypoglycemia less than normal; webbing of the neck; cubitus valgus; narrowing of the visual fields; supposed existence of aortic coarctation.

*Case 7.*—L. R. O., 24 years of age, entered the hospital because menstrual periods had never occurred and on account of her short stature. There was no development of the mammary glands. Pubic and axillary hair appeared at normal age. On some occasions she believes she had hot flashes which appeared spontaneously and lasted a very short



time. She has always been small. She attended school, having always been rather a bad pupil. Family history not available, as she has not known her parents. She only remembers having suffered in her infancy from measles and whooping-cough, and in 1929, she was obliged to enter a hospital for one month on account of pleurisy with effusion.

**PHYSICAL EXAMINATION:** Height 148 cm.; span 153½ cm.; weight 36.5 kg.

Pilous system: Scanty axillary and pubic hair.

Skeleton: Fingers and toes with trend to arachnodactylia. Very marked bilateral hallus valgus. Pronation of the left foot. Pes cavus at rest.

Mammary glands: infantile. Lack of development of areolae and nipples.

Circulatory system: blood pressure 110/70 (Baumanometer). The remainder normal.

Genitalia: infantile vulva, large labia minora at the prepuce of the clitoris; large clitoris.

Vaginosecopy: pink vaginal mucous membrane; the mucous membrane of the cervix is smooth with some irregularities which seem to be cystic.

Röntgenography showed generalized osteoporosis, epiphyses not fused and bone age of less than 19 years.

**LABORATORY DATA:** Hypophyseal follicle-stimulating-hormone (F.S.H.).

Nov. 3, 1945, positive for 96 mouse units in the urine per 24 hours.

17-ketosteroids: May 8, 1946, 12.1 mg.; May 13, 10.5 mg.

Carbohydrate metabolism: Exton-Rose test: fasting 98 mg. per cent; after 30 min. 103 mg.; after 60 min. 160 mg.

Insulin tolerance curve: Fasting 74 mg. per cent; after 20 min. 40; after 30 min. 44; after 45 min. 57; after 60 min. 69; after 90 min. 66; after 120 min. 72 mg. per cent.

Blood cell count: normal.—Urea: 27 mg. per cent.

Wassermann and Kahn: negative.—Cholesterol 212 mg. per cent in the blood.

Urine: normal.—Creatine in the urine: 99 mg. per 24 hours.

Creatine + creatinine: 99 mg.

Basal Metabolic Rate: +13 per cent. Vaginal smears: atrophic.

**OCULAR FUNDUS:** Normal except for a slight flexuosity of the arterial veins. The papilla of the right eye slightly raised at the inferior border, but it is felt that this is due to an arterial vein forming an angle at that level.

**VISUAL FIELDS:** Concentric and irregularly narrowed, more for red and green than for white.

**SURGICAL COMPROBATIONS:** An operation was undertaken for an appendicular crisis, disclosing very slender broad ligaments and a very small uterus, 4 cm. high; the round ligaments were atrophic; the tubes and pavilions very small. At the site of the ovaries, two whitish cords, ½ cm. in diameter, were observed. The right adnexa was removed.

**HISTOLOGICAL EXAMINATION:** (Dr. Colillas): In the ovarian portion cortical substance of regular thickness without any specific element of ovarian parenchyma; medullary zone richly irrigated by small congested veins. Some Walthard's inclusion, Very rudimentary tube. Müllerian residuals. Differentiated elements of the ovary are not noted.

**HISTOLOGICAL DIAGNOSIS:** Rudimentary ovary.

*Summary of Case 7.*—Diminished growth; proportions similar to those observed in eunuchoidism; primary amenorrhea; lack of mammary glands; diminished pubic and axillary hair; hypoplastic vulva; infantile uterus; atrophic vaginal smears; increased urinary gonadotropins (F.S.H.); slightly diminished 17-ketosteroids; diffuse osteoporosis;

diminished response to insulinic hypoglycemia. Very marked concentric narrowing of the visual fields for white and even more accentuated for red and green. The histological study disclosed the lack of characteristic ovarian elements.

*Case 8.*—*M. C.*, 24 years of age, entered the hospital on account of her short stature and because she has never had bloody discharges from her genital organs.

She has always been undersized as compared to her companions of the same age. Actually she does not suffer from any disorder.

At the age of 6, she had measles, and at 8, whooping cough. She attended only the first grade in school, which she had to repeat, and thereafter she abandoned school. Information about her family is not available because her parents interned her 7 years ago into a College-Asylum.

She has good appetite; her alimentation is normal.

**PHYSICAL EXAMINATION:** Stature 133½ cm.; span 139 cm.; weight 40 kg; well nourished; fatty tissue with feminine distribution. Her facial appearance corresponds to a person of mediocre intelligence.

Pilous system: fine and very scanty pubic hair, increased in quantity on the labia of the vulva. Axillary hair has not developed. Discrete hirsutism of the extremities.

Skeleton: harmonical; the third toe of the left foot is very short.

Mammary glands: breasts infantile with some fatty tissue; infantile nipples and areolae.

Circulatory system: Blood pressure: 115/60. The remainder without pathological alterations.

Genitalia: Hypoplastic vulva. Clitoris of normal size. Hymen preserved. A small stump is felt per rectum corresponding to the uterus; ovaries are not felt.

Of low intelligence, corresponding to a girl of 8, with very little instruction.

**ROENTGEN RAYS:** of the sella turcica: normal; teleroentgenography: cardiovascular shadow small in relation to the thorax, middle arch prominent. Hands: the conjugation cartilages of the phalanges and the metacarpal bones persist. Bone age: corresponding to 18 years.

**LABORATORY DATA:** Hypophyseal follicle-stimulating-hormone (F.S.H.).

May 7, 1946, positive for 96 mouse units in the urine per 24 hours.

May 14, 1946, positive for 96 mouse units in the urine per 24 hours.

17-ketosteroids: May 13, 1946: 19.3 mg. in the urine per 24 hours.

Blood cell count: normal.—Urea: in the blood: 30 mg. per cent.

Glycemia: 98 mg. per cent.—Cholesterol in the blood: 192 mg. per cent.

Wassermann and Kahn: negative.

Urine: normal. Density: 1.024.

Carbohydrate metabolism: Exton-Rose test: fasting 80 mg. per cent; after 30 min.

163 mg. per cent; after 60 min. 160 mg. per cent.

Vaginal smears: atrophic.

**VISUAL FIELDS:** Slight retraction of the external isopter. Ocular fundus normal.

**SURGICAL OPERATION:** An exploratory laparotomy disclosed a very small uterus, 3½ cm. long and 1½ cm. wide, very tenuous broad ligaments, very thin round ligaments and tubes; at the site corresponding to the ovaries, only two whitish cords of a small diameter and about 3 cm. long are observed. Material for biopsy is removed.

**HISTOLOGICAL EXAMINATION (Dr. Colillas):** The biopsy showed a non-specific stroma,

different from that of the ovary. In none of the sections could differentiated ovarian elements be found.

#### HISTOLOGIC DIAGNOSIS: Rudimentary ovary.

*Summary of Case 8.*—Pronounced decrease in stature; proportions of the skeleton similar to those in eunuchoidism. Bone age retarded, primary amenorrhea; infantile mammary glands; very scanty pubic hair; lack of axillary hair. Determination of the F.S.H.: titers higher than normal. 17-ketosteroids, normal values. Rudimentary ovaries. Marked hypoplasia of the external genitalia.

#### DISCUSSION

Table 2 shows the principal characteristics of the patients whose clinical histories have been presented. A study of this table reveals that these ob-

TABLE 2

Name	Age	Height in cm.	Span in cm.	Bone age	Beginning of retar- dation of growth	Menstru- ation	Hair		Mam- mary glands	F.S.H.	17-keto- steroids	Bone alterations
							Pubic	Axil- lary				
T. Case 1	20	166	166	<18	No retarda- tion	0	+	0	0	+96 mouse units	7.21 mg	Osteoporosis carpus and knee
T.R. Case 2	18	146	152	<18	Before the age of 6	0	+	+	0	+96 mouse units	24.06 mg	Osteoporosis of spine vertebral chondrodys- tr.
L. Case 3	20	135	140	<18	Since the infancy	0	+	+	0	+96 mouse units	6.66 mg	Generalized osteoporosis vertebral chondrodys- tr.
V. Case 4	17	140	142	18	Before the age of 6	0	++	+*	+*	+96 mouse units	5.88 mg	Generalized osteoporosis
F. Case 5	22	136	142	<20	Has always been short	0	+	+	+*	+96 mouse units	11.29 mg	Generalized osteoporosis, chondrodys- trophies
J.J.M. Case 6	17	131	127	17	Since the age of 3	0	0	0	0	+96 mouse units	6.03 mg	Vertebral osteoporosis, chondrodys- trophies
O. Case 7	24	148	153	>19	Has always been short	0	+	+	0	+96 mouse units	10.45 mg	Generalized osteoporosis
C. Case 8	24	133½	139	>18	Has always been short	0	+	0	0	+96 mouse units	19.27	

\* Has been treated with estrogens or gonadotropins.

servations correspond to the syndrome of rudimentary ovaries, a fact which has been corroborated in six of them by histological studies on tissues of the genital organs removed during surgical exploration.

The age of these patients ranged from 17 to 24 years, with one exception. All of them showed retardation of growth since childhood, which was well marked at the age of puberty. Such retarded growth (see Table 1) may be appreciated from the statures of the patients, since they varied from 131 to 148 cm., in height. *Case 1* had a normal height: 166 cm., (Fig. 14).

It is difficult to settle correctly the period of life at which this disorder starts. In the majority of patients it seems to be during the school age. In *case 6*, a marked retardation of growth began at the age of 3.

According to the observations made, it is our belief that this syndrome is not necessarily accompanied by short stature, though this occurs in the

TABLE 2—Continued

Deformities	Ocular abnormalities	External genital organs	Vaginal smears	Genital examination	Biopsy
Cubitus valgus. Partial syndactylism of toe	Visual fields normal	Hypoplastic. Small clitoris	Atrophic	Hypoplastic uterus and tubes; at the site of the ovaries two whitish cords	Rudimentary ovaries
Cubitus valgus; talipes	Narrowing of the visual fields	Hypoplastic. Great clitoris	Atrophic	Hypoplastic uterus and tubes; at the site of the ovaries two thin cords.	Rudimentary ovaries
Talipes	Narrowing of the visual fields	Hypoplastic. Normal clitoris	Atrophic	Hypoplastic uterus and tubes; at the site of the ovaries very thin cords	Rudimentary ovaries
Cubitus valgus; talipes		Hypoplastic; clitoris normal	Atrophic	Small uterus. Hypoplastic tubes. Two cords at the site of the ovaries	Rudimentary ovaries
Cubitus valgus	Normal visual fields	Hypoplastic. Normal clitoris	Atrophic	Small uterus felt per rectum. Vaginocopy: small cervix; red mucous membrane	
Cub. valg. Sphynx' neck; aortic coarctation	Narrowing of the visual fields	Marked hypoplasia, small clitoris	Atrophic	Small uterus felt per rectum. Vaginocopy: small cervix; irregular mucous membrane	
Talipes	Marked narrowing visual f.; more pronounced for red and green	Hypoplasia, great clitoris	Atrophic	Small uterus; hypoplastic tubes. Ovaries: two whitish cords.	Rudimentary ovaries
	Narrowing of the visual fields	Hypoplasia, normal clitoris	Atrophic	Very small uterus. Two filiform formations.	Rudimentary ovaries

large majority of the cases. In spite of the fact that Albright, Varney and their respective co-workers ascribe a primordial importance to the retardation of growth, it must be kept in mind that the first author mentions in his publication (1942) two patients,<sup>2</sup> whose stature was normal and, furthermore, that in the cases observed by Sellheim (1924) (28), Kuliga (1930) (14) and Meyer (1931) (16), the body heights of the patients were 161, 176 and 169 cm., respectively.

These circumstances have influenced us in calling the syndrome "Rudimentary ovaries with estrogenic insufficiency and increase in gonadotropins," whereas Albright and his collaborators, as well as Varney and his co-workers include the retardation of growth in the titles of their respective papers.

Although up to the present time the pathogenesis of the retarded growth in this syndrome has not been elicited, there are several theories for its explanation. Because of the fact that the epiphyses in these patients fuse later than normally, it is impossible to ascribe this disorder to a precocious closure of the conjugation interspace. The retardation of growth would appear to be logically related:

- 1) to the failure of some of the usual growth stimulants, or
- 2) if these stimulants act in a normal way, the conjugation cartilages do not respond as they do usually.

Accepting both hypotheses, this disorder could be a result of solely genetic factors or of secondary endocrine alterations as a result of ovarian hormone deficiency. Varney, Wilkins and their respective co-workers (1942) admit the genetic factors, whereas Albright and his group favor the hypothesis that the retardation of growth is due to a diminution of the cortico-adrenal function. This diminution would appear to be a consequence of a reduced hypophyseal stimulation which, in turn, is the result of a diminution in estrogen.

Other fundamental manifestations of this syndrome are hypoplasia of the genitalia and primary amenorrhea. All our patients showed modifications of the sexual characteristics. None of them had had spontaneous menstruation; in two (*cases 1 and 5*) blood losses from the vagina took place as a consequence of treatments not clearly specified. The mammary glands were infantile, with lack of glandular tissue and without development of the areolae and nipples. In two patients (*cases 4 and 5*) some glandular development was observed as a result of treatments with estrogenic substances.

The external genitalia were hypoplastic in all the cases. The clitoris was normal in *cases 1, 3, 4, 5, 6 and 8*, and increased in size in *cases 2 and 7*.

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<sup>2</sup> One of us (de la Balze) has had the opportunity to observe these patients.

On the majority of patients vaginoscopies had been performed with Luy's urethroscope; it was noted that the vaginal mucous membrane was normal and the cervix very small, as well as the external orifice. In one of the patients (*case 6*), the mucous membrane of the cervix was infantile, whereas in the others it was smooth, similar to that observed after puberty. Vaginal smears performed on different dates were atrophic in type, disclosing lack of estrogenic stimulation.

These findings, typical of childhood, in these patients, prove that changes in the secondary sexual characteristics such as are ascribed to ovarian activity, had not taken place at the time of puberty.

As to the pubic and axillary hair, in *case 6* only was the complete lack of pubic and axillary hair noted. In 6 patients the pubic hair was scanty; axillary hair was absent in *cases 1* and *8*.

From the foregoing it may be seen that same discrepancy exists between the secondary sexual characteristics and the development of pubic and axillary hair. In order to explain this phenomenon, the theory advanced by Albright and his co-workers (1942) deserves mention. These authors consider that ovarian function *per se* neither determines the development nor governs the ulterior growth of the sexual hair, but that cortico-adrenal androgens are responsible for these modifications.

Increase in urinary gonadotropins has been disclosed in all our patients, and on repeated assays, higher titers for the hypophyseal gonadotropins in urine have been found.

No doubt, without the biological tests, it would be difficult to establish the existence of the syndrome under discussion. One must keep in mind that patients with this syndrome suffer not only from primary amenorrhea but, in addition, from all the other manifestations of estrogenic insufficiency; and under the circumstances it is interesting to find out whether estrogenic insufficiency is primarily ovarian in origin or secondary to hypophyseal gonadotropic insufficiency.

If estrogen insufficiency is primarily ovarian in origin, the F.S.H. will be increased in the urine, either because the hypophysis, in the absence of the effect of ovarian hormones "becomes unbridled," producing an exaggerated quantity of them (Albright and others), or because, when an end-organ, in this case the ovary, does not exist, the gonadotropins will be excreted in the urine in increased quantities, (Heller and others). If it were a question of a primary hypophyseal lesion, a lack or decrease of the F.S.H. in the urine would be noted. We determined the amount of estrogens in urine in only 2 patients (No. 2 and 3). In both it was diminished, i.e., less than 24 i.u. in the urine per 24 hours.

The amenorrhea, the atrophic vaginal smear, the lack of development of the mammary glands, nipples and areolae, as well as the hypoplastic

external genitalia can be reasonably ascribed to estrogenic insufficiency.

In the cardiovascular system, the blood pressure was slightly increased in cases 2 and 3. The figures indicated by Mac Laren (1930) (15) have been used as normal values.

In one case (No. 6), a systolic murmur was heard at the apex of the heart, which was more intense in the left interscapulo-vertebral region without other elements to establish correctly the diagnosis of aortic coarctation, such as have been noted in several of the observations made by Albright and his co-workers (1942).

All our patients showed one or several abnormalities of their skeletal structure. We recall that while describing the disorders of growth, short stature has been mentioned in cases 2, 3, 4, 5, 6, 7 and 8. The proportions of the body were similar to those found in eunuchoidism, i.e., the span exceeded the height in cases 2, 3, 4, 5, 7 and 8. This is the result of late closure of the epiphyses of the long bones with respect to the skeleton. In all 8 cases a diffuse osteoporosis existed, predominately in the vertebrae and the ilium. According to the observations of Albright and his co-workers, this osteoporosis is similar to that observed in women after the menopause, and it probably should be ascribed to estrogen deficiency.

In five patients (cases 2, 3, 4, 6 and 7) it is interesting to note that the lesions of the epiphyses of the dorsal vertebrae are located mainly on the joint borders, which are irregular and deformed (see the roentgenographs). Such alterations have been called "epiphysitis," but might more correctly be called chondrodystrophia, on account of their anatomical characteristics.

The roentgenographic study in five of our patients (cases 1, 3, 5, 7 and 8), disclosed a delay in bone age in relation to the chronological age. Keeping in mind that the ages of our patients ranged from 17 to 24, it is difficult to reach at the moment definitive conclusions concerning the extent of this delay. In cases 2, 4 and 6, though the bone age actually coincides with the chronological age, we are not sure whether or not in the future a delay will occur in the ossification.

In patients 2 and 3, an exaggerated articular lassitude was noted.

The following congenital abnormalities were disclosed: in cases 1, 2, 4, 5 and 6 there was cubitus valgus, observable in several of the photographs; pes cavus at rest was noted in cases 2, 3, 4 and 7, and the photographic proof of one of these patients has been included (Fig. 15).

Patient No. 6 reveals the abnormality called by us "Sphynx' neck." Turner described in 1938 a syndrome of infantilism with "webbed neck" and cubitus valgus. Very probably the cases presented by this author may be included in the syndrome under discussion because of the clinical char-

acteristics, although in none of them were the urinary gonadotropins determined, or the histological study of the ovaries undertaken.

As to the metabolism of the carbohydrates, it was noted (see Table 3) that among the six patients (*cases 1, 2, 3, 5, 6 and 7*), on whom the insulin tolerance curve was performed, in three (*cases 2, 3, and 6*) failure to recover from the hypoglycemia induced by insulin was observed. This is shown by the samples removed after 120 minutes. This abnormality has been found



FIG. 15. Pes cavus at rest, corresponding to *case 3, R. L.*

in patients who have hyperinsulinism, total hypophyseal insufficiency and cortico-adrenal insufficiency.

In the cases presented, the 17-ketosteroid assay showed reduced values. In patients *1, 3, 4, 5, 6 and 7* (see Table 2), it ranged from 5.88 to 11.25 mg., normal values in *case 8*, and showed increased values (24.06 and 28.02 mg.) in patient *2*. It might be recalled that the latter patient showed in

TABLE 3. INSULIN TOLERANCE TEST

Observation	No. 1	No. 2	No. 3	No. 5	No. 6	No. 7
Fasting	99	98	94	82	86	74
20 min.	52	54	54	56	43	40
30 min.	53	46	71	47	54	44
45 min.	88	60	56	68	50	57
60 min.	99	83	58	79	58	69
90 min.	106	89	74	79	58	66
120 min.	99	72	62	90	62	72



addition hirsutism and hypertrophy of the clitoris, and that the histological study of the tissue removed for biopsy from the site corresponding to the ovary revealed an inclusion of cells, possibly adrenal in origin. This observation would support the hypothesis of the existence of a direct relation of the increase of the 17-ketosteroids to hirsutism.

It must be kept in mind that the neutral 17-ketosteroids in the urine seem to be in the female a product of the steroids metabolism produced by the adrenal cortex. The possibility that the ovary could contribute to the production of these steroids must be discarded in our cases, because of the rudimentary condition of this organ.

While considering this question, Albright thought that the low titers of the 17-ketosteroids might be the result of adrenal cortex hypofunction which, in turn, is the result of the lack of estrogenic action.

*Syndrome of Adaptation.*—Phase of the *Alarm-Reaction*. Thanks to Selye's research (1946) (26) the existence of a syndrome of reaction on the body to the noxious stimuli has been revealed, the so-called "Adaptation-Syndrome." In relation to this it must be recalled that Forbes (1942) (8) showed that at the beginning of a febrile condition, after certain surgical operations and, in general, accompanying an organic lesion, the urinary 17-ketosteroid assays show certain modifications. During the first days, they are increased above normal, falling thereafter below the normal level. Similar results have been obtained by other investigators, (Stevenson, Schenker and Browne, 1944 (30); Cope, Nathanson, Rourke and Wilson, 1943 (6)). In general, it is accepted that these modifications in the 17-ketosteroid values represent one of the manifestations of the so-called alarm-reaction.

In three of our patients the behavior of the 17-ketosteroids was studied during the days following surgical procedure. The patients responded with a curve similar to that of normal persons under similar conditions (Forbes, 1942) (see Table 4). It can be seen that the only difference is due to the fact that the pre-operative values were lower than those observed in normal individuals.

As a result of these findings it may be said that presumably the mechanisms involved in this reaction (de la Balze, 1946 (3)) show the same response in patients with the syndrome of rudimentary ovaries as in normal individuals.

The visual field was tested in seven of the patients (Dr. R. Gil). In two cases (1 and 5) the findings were normal. In four cases (2, 3, 6 and 8), a slight concentric narrowing of the isopters, corresponding to the perimetry and visual fields, was noted. In case 7 (see field chart No. 1), the narrowing is very accentuated, particularly in the internal isopters. On

this patient, a study of the visual fields with respect to the red and green colors was made revealing a more marked narrowing of it than for the white color. In order to determine the cause of this disorder, an encephalogram with injection of air was taken (Dr. Dowling); several films showed normal pictures.

The visual field disorders observed on various patients who had the syndrome of rudimentary ovary could perhaps be ascribed to a process of arachnoiditis or a condition of another nature compressing the optic ways

TABLE 4

Date	Levels of 17-Ketosteroids		
	Case No. 1	Case No. 3	Case No. 4
Previous to operation	7.2 mg.	8.9 mg.	5.9 mg.
First day after operation	14.9 mg.	9.2 mg.	16.2 mg.
Second day	12 mg.	14.6 mg.	18.8 mg.
Third day	—	13.9 mg.	16 mg.
Fourth day	5.9 mg.	5.5 mg.	9.4 mg.
Fifth day	—	—	4.5 mg.

Study of the 17-ketosteroids previous to an operation and during the first five days after that operation. The levels are similar to those observed in normal individuals, but the initial and terminal values are lower.

in the neighborhood of the chiasm. In spite of this hypothesis, in one of our cases presenting the most accentuated lesions the encephalogram disclosed normal findings.

Six patients (cases No. 1, 2, 3, 4, 7 and 8) underwent laparotomies on account of intercurrent conditions; these interventions allowed the study of the internal genitalia and the extirpation of specimens for biopsy, (see Table 2). The macroscopical examination of these organs revealed in all patients a considerable delay in development. The uterus, tubes, broad and round ligaments were all reduced in size and hypoplastic in character. At the site corresponding to the ovaries, two whitish cords, increased in length and small in diameter, were encountered, (Fig. 16). In cases 5 and 6, a very small uterus was felt per rectum, but it was not possible to feel the adnexae.

#### DIAGNOSIS

The patients consult the physician because bloody discharge from the genital organs has not appeared although they have passed puberty, some of them several years ago. Furthermore, in the majority of the patients, but not in all of them, an evident delay in growth is noted.

On physical examination, in addition to the anamnestic data mentioned, the very marked failure in the development of the sexual characteristics is noted as an expression of estrogenic insufficiency. In all the cases the urinary gonadotropin titers are above the normal level.

Based upon these clinical findings, it is possible to establish correctly the diagnosis of syndrome of rudimentary ovary.

Notwithstanding, before this conclusion is reached, it is necessary to find out if the genital organs have been removed during the prepuberal



FIG. 16. Drawing made during the surgical intervention of case 3, R. L. The small size of the uterus and the hypotrophy of the round ligaments, tubes and broad ligaments are observed. Underneath the pavilions two whitish cords are seen corresponding to the rudimentary ovaries.

age for any condition requiring castration. This situation can be ruled out by the physician if a careful interrogation and a watchful physical examination are undertaken.

The diagnosis of this syndrome can be established by means of the anamnestic data, the physical examination and the determination of the urinary gonadotropin titers. The cases observed by Albright (1), Varney (33), and our own eight cases corroborate this statement.

Neither a laparotomy, which allows the observation of the development of the internal genitalia, nor the histological study of the tissues removed for biopsy are essential to establish the diagnosis, though they confirm it undoubtedly, thus giving an anatomical basis.

Once the characteristics of the syndrome are established and the disorders from which the patients suffer are studied, it is imperative to differentiate the clinical picture from that of other conditions disturbing growth and modifying the development of the sexual organs.

We refer first of all to the infantilisms which are a consequence of intense and prolonged nutritional deficiencies in children. In such cases the anamnestic data disclose the existence of evident quantitative and qualitative nutritional insufficiencies. The latter have not occurred in our cases.

Nor is it a question of infantilism accompanying severe condition of the digestive tract, kidneys and cardiac system, or those observed in certain illnesses with hepatosplenomegaly, in Schüller-Christian's syndrome, in Lobstein-Eddowes' disease, in Rothmund-Werner's syndrome or in parathyroid dwarfism; these affections show signs and symptoms which allow their differentiation (del Castillo and co-workers, 1944 (5)).

The possibility of a hypothyroid infantilism can be ruled out, since our patients showed neither the clinical manifestations nor the corresponding findings of the biological tests or of the complementary examinations which would allow corroboration of the presence of this condition.

The most difficult differential diagnosis to be made, in these cases of rudimentary ovaries which present diminution of growth, is that of the so-called "hypophyseal dwarfs."

In order to facilitate the differentiation between the syndrome under discussion and hypophyseal dwarfism, the following comparative Table has been made:

<i>Rudimentary ovaries</i>	<i>Hypophyseal dwarfism</i>
1) Women of short stature.	1) Dwarfs.
2) Infantile mammary glands and genital organs.	2) The same.
3) Development of pubic and axillary hair.	3) Lack of pubic and axillary hair.
4) Well nourished and strong.	4) Weak and easily tired.
5) Bone age some years retarded.	5) Very marked delay in bone age.
6) Late closure of the epiphyses.	6) Lack of closure of the epiphyses.
7) Very frequently vertebral chondrodystrophia.	7) The same.
8) Follicle-stimulating-hormone increased in the urine.	8) Lack of follicle-stimulating hormone.
9) 17-ketosteroid somewhat diminished.	9) 17-ketosteroid considerably diminished.

- |   |   |
|---|---|
| 10) Normal insulin curve.   | 10) There is no response to insulinic hypoglycemia.       |
| 11) Congenital abnormalities; among them aortic coarctation and "Sphynx" neck." | 11) Not observed.   |
| 12) Diffuse osteoporosis and early senility.                                    | 12) Not observed.   |
| 13) Normal sella turcica.   | 13) Pathological modifications may be observed.           |
| 14) Visual fields: some functional alterations.                                 | 14) Abnormalities in the presence of a neoplastic lesion. |

It is necessary to point out clearly that the differential features indicated in this Table refer particularly to the cases of dwarfism and infantilism associated with a hypophyseal lesion of the organic destructive type; in the so-called "hypophyseal dwarfs" without apparent hypophyseal lesions, the differentiation would be very difficult if biological methods were not available which allow urinary gonadotropin assays.

We insist on the fundamental fact that in the case of a syndrome such as results from an organic hypophyseal lesion, the urinary gonadotropin assays show titers lower than the normals whereas in the cases under discussion, the determinations always showed titers above the normal levels. This fact, established in human pathology is, as has been mentioned, in accordance with experimental research; a vast literature exists on the subject.

#### SUMMARY

Eight cases corresponding to the syndrome designated as "Rudimentary Ovary with Estrogenic Insufficiency and Increase in Gonadotropins" have been presented.

In six cases the histological study of the ovaries and the macroscopical examination of the internal genital organs has been performed.

These new observations help to establish in a categorical manner the existence of this new syndrome described for the first time and simultaneously by Varney, Kenyon and Koch (1942) and Albright, Smith and Fraser (1942).

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# TREATMENT OF CARCINOMA OF THE HUMAN BREAST WITH TESTOSTERONE PROPIONATE:<sup>1</sup> A REPORT OF FIVE CASES

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THE relationship of sex hormones and neoplasms has been the stimulus for a great deal of investigation in recent years. Loeb and his associates (8, 9) using mice as experimental animals, demonstrated a definite relationship between estrogenic hormone and mammary carcinoma. By means of castration they were able to decrease the incidence of spontaneous carcinoma of the breast in mice. Lacassagne (6) in 1936 and Suntzeff, et al. (12) in 1936 lowered the tumor age and increased the tumor incidence of breast carcinoma in various strains of mice by using estrogenic substance. Clinically, withdrawal of estrogen by surgical or roentgen castration as an adjunct in the treatment of carcinoma of the human breast is an old and acceptable procedure (2).

Following Lacassagne's (7) pioneer work in the use of testosterone propionate in mammary carcinoma of mice, Nathanson and Andervont (11) using the androgen in larger doses, decreased the incidence of mammary tumors in a high tumor strain of mice. Ulrich (13) applied this form of therapy to humans and obtained a favorable response in two patients with inoperable mammary carcinoma. Loeser (10) treated five cases of recurrent carcinoma of the breast with large doses of androgen and noted improvement and no further recurrence for as long as five years. Fels (5) described three cases with good results in one and questionable results in the other two. Boger (3) reported one case treated with a combination of surgical castration and methyl testosterone orally, obtaining relief of pain and recalcification of osteolytic lesions. Adair and Herrmann (1) added eleven cases to the literature with favorable results in four. Farrow and Woodward (4) using smaller doses of androgen in a series of thirty-three patients obtained equivocal results. We wish to report five cases treated with testosterone propionate<sup>2</sup> in which results are sufficiently promising to warrant further investigation.

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## CASE REPORTS

*Case 1: M. H.*, a colored female, aged 46, was admitted to the Clinic in January, 1941. There was a history of a small boil developing about the right nipple while nursing her first child twenty-seven years previously. This drained spontaneously and in healing left a small mass. Two years before admission this mass began to increase slowly in size. She had had two normal pregnancies. At the time of admission she was menstruating regularly.

Physical examination disclosed a hard mass in the right breast seven centimeters in diameter, fixed to the skin, but movable over the chest wall. The nipple was retracted. The skin over the tumor was ulcerated. The axillary nodes were enlarged and very hard. No other metastases could be found by physical or x-ray examination.

The patient refused operation. Upon her return thirteen months later, the primary tumor had increased in size to eight by ten centimeters and ulcerated through the skin. The ipsilateral axillary nodes had also increased in size. The tumor was still movable over the chest wall, and no distant metastases were found. A radical mastectomy was performed at which time the tumor was found to encase the axillary structures and could not be completely removed. A month later a course of deep roentgen therapy was given to the right breast and axillary area. She was also given a sterilizing dose of roentgen therapy to the ovaries. Twenty-three months after surgery, two small recurrences were found adjacent to the operative scar. These responded to roentgen therapy but four months later recurrences reappeared in the same area, and in the axilla. No further treatment was instituted and in the following year she lost forty pounds in weight, developed pleurisy with a small amount of effusion in the left chest, and skeletal metastases to the left sixth rib, both ilia, right acetabulum and left ischium. She had vague gastrointestinal disturbances and an enlarged liver. There were many skin metastases around the operative site, some of which had ulcerated.

Forty months after operation and six years after the appearance of the primary tumor, she was extremely emaciated, and bedridden because of the severe pain in her right hip. Testosterone propionate therapy was instituted. She received 500 milligrams in a period of five weeks, given in 25 and 50 milligram doses intramuscularly. We attempted to give the injections at three day intervals, but this was not always possible. After receiving 250 milligrams there was marked improvement in symptoms of pain to the extent that the patient was able to be up and to make trips out of town. At this time she also complained of soreness in the metastatic areas. Roentgenograms two weeks after cessation of treatment showed no change in skeletal metastases despite alleviation of pain. These were repeated three months later and were reported as follows: "In the pelvis can be seen malignant metastases in both ilia lateral to the sacro-iliae joints, in the acetabular portion of the right ilium, in the right acetabulum, in the greater trochanter of the right femur, and in the body of the left ischium. There has been a definitely increased calcification in all but the focus in the acetabular portion of the right ilium and in the trochanter of the right femur. It appears as though the lesion in the left ilium is larger than it was in June, but there is a definitely increased density and a network of dense calcification. No new lesions are visible."

Subsequent roentgenograms continued to show recalcification of the metastatic lesions until seven months after cessation of her first course of testosterone. At this time there was a definite increase in size and amount of bone destruction of the osseous lesions. In the twelve months following her first course of treatment, she received 375 milligrams of testosterone propionate in 25 and 50 milligram doses at intervals which

were irregular because of our inability to control her appearance at the Clinic. She gradually became worse and when last seen was considered to be in the terminal stage of carcinomatosis with ascites.

Three months after cessation of her first course of testosterone, three control determinations of serum calcium were 11.8, 11.1, and 9.3 milligrams per cent. She was then given 125 milligrams of testosterone in two weeks, and her serum calcium on the day of the last injection was 15.3 milligrams per cent. Five months later, with no intervening treatment, the serum calcium was again 11.8 milligrams per cent. After another course of 150 milligrams of testosterone in six days, her serum calcium was 10.6 milligrams per cent on the second day after the last injection. In spite of the fluctuation of the serum calcium, inorganic phosphorus and alkaline phosphatase values determined simultaneously were normal at all times. Routine urinalyses showed no abnormalities. She had a persistent leucopenia, but no abnormalities in the differential count. The erythrocyte count was normal.

*Case 2: B. N.*, a colored female, deaf mute, aged 36 was admitted to the Clinic in February, 1946. She gave a history of a mass in her left breast which had been growing rapidly for one year. She had had two normal pregnancies and was menstruating regularly at the time of admission.

Physical examination disclosed a hard irregular mass in the left breast six centimeters in diameter. It was not fixed either to the skin or chest wall. There were enlarged nodes, hard when palpated, in both axillae. A biopsy of the primary tumor and both axillary nodes showed carcinoma. There were no skeletal metastases. The hemogram and urinalysis were within normal limits.

Testosterone therapy was instituted, 25 milligrams was given intramuscularly for the first dose and then 50 milligrams every three days. Three weeks later she developed a generalized pruritus with some eczema formation. The dosage was reduced to 25 milligrams every three days and palliative topical applications were used on the skin lesions. There was no history of previous pruritus and no etiology could be found to explain it except perhaps the testosterone or its oil base. With continued therapy, however, it cleared up and then the dosage of androgen was increased to 100 milligrams per week given in 25, 25, and 50 milligram doses. Therapy was stopped empirically in 105 days after a total dosage of 1425 milligrams.

Reaction to the medication was nausea, flushing of the skin and possibly pruritus. She complained of "drawing" pain in her left breast and dull pain in legs and back, but these were not severe enough to incapacitate her. She developed an increased hirsutism of the lower extremities and diminution in size of the uninvolved breast. She menstruated three days before the onset of therapy and again six weeks later during therapy. There were no further menstrual periods until four weeks after cessation of treatment. Following this she was given a castration dose of roentgen irradiation.

Three to four months after treatment was stopped, her breasts began to increase in size but there was no change in the hirsutism. Her weight showed a small but definite increase at the beginning of treatment, maintained itself until therapy was stopped, and then gradually decreased (see Figure 1). The serum calcium was initially 12.4 milligrams per cent, and showed no significant change during the course of treatment. The alkaline phosphatase fluctuated between 1.6 and 6.0 Bodansky units and the serum inorganic phosphorus between 2.75 and 5.10 milligrams per cent. No abnormal changes in the hemograms and urinalyses were noted.

In the eight months prior to castration, there had been a definite increase in the size

of the primary tumor with fixation to the skin in the anterior axillary fold. The regional axillary metastases also increased in size but no discernible distant metastases developed. No systemic or symptomatic changes in the patient have been noted.

*Case 3: F. D.*, a white female, aged 55, was admitted to the Clinic in March, 1946. She gave a history of a hard mass which had been present in the right breast for several years. Four months prior to admission she noticed two small "knots" which seemed to be attached to the mass and to the skin. These soon broke down and discharged a thick yellowish material. She believed she had lost 30 to 35 pounds during the previous year.

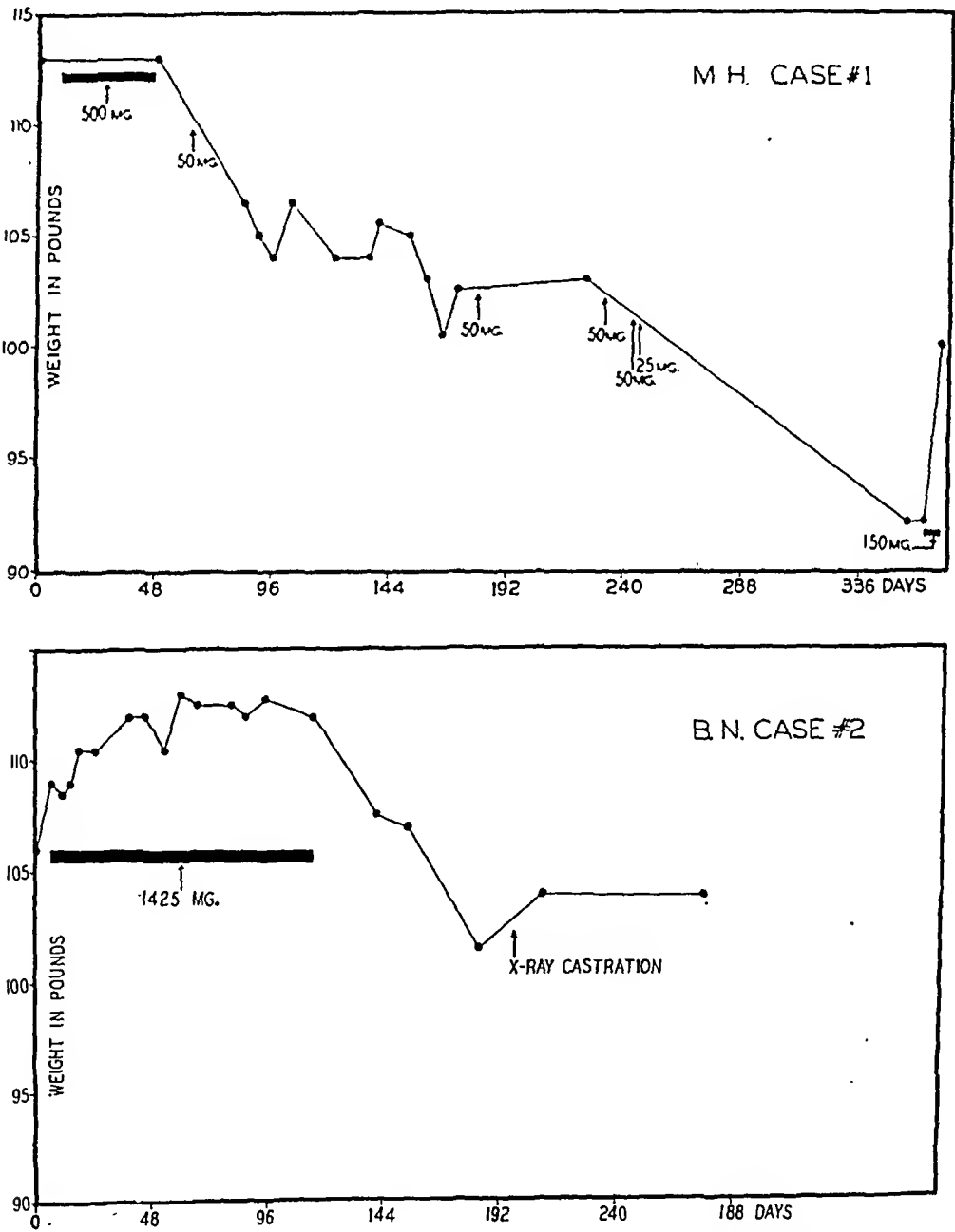


FIG. 1. Graphic representation of the effect of testosterone propionate (Oreton) on the weight of patients treated for carcinoma of the breast.

Physical examination revealed the entire right breast to be replaced by carcinoma measuring about 13 by 8 centimeters. The tumor was firmly fixed to the chest wall and had invaded the overlying skin forming an ulceration five centimeters in diameter. There was a separate stony-hard mass above the breast fixed to the chest wall just to the right of the angle of Lewis. The right axillary nodes were enlarged and hard. Biopsy of the ulcerated area revealed carcinoma. Roentgenograms showed metastases to the lungs and a sclerosing lesion of both sacro-iliac joints. An incidental finding was a nodular non-toxic goiter which had been present for twelve years.

She received 550 milligrams of testosterone propionate in 25 and 50 milligram doses over a period of 75 days. No improvement was noted in the primary tumor or the metastases and she became progressively weaker. Because of this and the fungating nature of the lesion, palliative roentgen therapy was given to the primary lesion. The patient did not improve and died at home seven months after she was first seen. Permission for autopsy was not obtained.

No reactions to the androgen were noted, nor any effect on the hemogram, serum calcium, inorganic phosphorus, or alkaline phosphatase. Her goiter did decrease in size during treatment.

*Case 4: D. W.*, a white female, aged 42, was admitted to the Clinic in February, 1945. She had first noticed a small lump in her left breast fifteen months prior to admission. Two months after the appearance of the first lesion, a small mass appeared in the skin of the right lumbar region. At this time a left simple mastectomy and removal of the skin nodule was done at another hospital. Both lesions were diagnosed as carcinoma. Her post-operative course was uneventful for the first five months, when she developed pain in the left axilla and both sides of the neck. A radical dissection was then done on the left axilla at another hospital. Following the operation she had pain in her neck and left arm, which at the time of her admission to our Clinic was so severe that she could not move these parts and was confined to bed.

Physical examination revealed skin metastases along the healed operative scar. The supraclavicular and cervical lymph nodes bilaterally were matted together in almost solid sheets, forming a continuous mass of firm tissue extending from the supraclavicular fossa to the tip of the mastoid on both sides. The skin over these areas was edematous and thickened. The left arm was edematous with marked tenderness on pressure over the middle third. The axillary nodes were involved bilaterally. The right breast contained no tumor. There was a single metastasis recurrent in a small scar in the right lumbar region. Roentgenograms of the skeleton were suggestive of a destructive process in the head of the left humerus. Hemograms, urinalysis and blood chemistry studies revealed nothing of significance.

She was admitted to the hospital and given 25 milligrams of testosterone propionate daily for a total of 300 milligrams. Fifty milligrams three times weekly was then prescribed and she was sent home. A total of 750 milligrams of testosterone was administered in 35 days. There resulted a very noticeable improvement of the pain in her neck and left shoulder but she continued to get worse and died 110 days after the onset of therapy. She frequently complained of sudden swelling and fullness in the posterior triangles of her neck occurring from 12 to 24 hours after the administration of the 50 milligrams of testosterone. The attending nurse stated that at these times an increased edema of the neck was visible. This would subside in about 12 hours. We have no explanation for this phenomenon. An autopsy was obtained and the diagnosis was recurrent carcinoma of the left breast with metastases to both axillae, right breast, cervical lymph nodes bilaterally, mediastinal lymph nodes and liver, and bronchopneumonia. Micro-

scopic study did not reveal any changes in the malignant cells which we could attribute to the hormonal therapy. However, she had not received testosterone for two months before death.

*Case 5: C. K.*, a colored female, aged 45, was admitted to the Clinic in April, 1946. She had had a "knot" present in her left breast for twenty months. The tumor increased in size and three months before admission her right shoulder became painful.

Physical examination revealed an acutely ill, cachectic patient, obviously in pain. Any movement of her head, trunk or extremities elicited severe but vaguely localized pain. Movement or pressure of the upper end of the right humerus invoked excruciating pain. A satisfactory physical examination and roentgen study were impossible. In the left breast was a stony-hard mass six centimeters in diameter fixed to the skin but not to the underlying structures. The left axillary nodes were enlarged, hard and matted together. She presented the picture of terminal carcinomatosis.

The hemogram and urinalysis were not unusual. The serum calcium was 14.6 milligrams per cent, the alkaline phosphatase was 1.8 Bodansky units. She was given 175 milligrams of testosterone propionate in a period of four days. The pain in the right shoulder improved to the extent that she could voluntarily move her right arm which heretofore she had been unable to do. She became moribund on the sixth hospital day and died on the eighth day. An autopsy was performed and the diagnosis was carcinoma of the left breast with metastases to the left axillary lymph nodes, head of the right humerus, liver, lungs and occipital bone with complete destruction of the bone and extension into the cerebellum, bilateral pulmonary edema, healed tuberculosis of the lungs and right axillary nodes, fibrous pulmonary adhesions, fibrous peritoneal adhesions, and leiomyomata uteri. Microscopically, we were unable to find any change in the malignant cells which we could ascribe to the hormonal therapy.

#### COMMENT

All the above cases were treated with testosterone propionate in sesame oil (Oreton) by intramuscular injections. It was given in doses of 25 and 50 milligrams per injection for a total dosage of from 175 milligrams in four days to 1425 milligrams in 105 days. We tried to maintain the weekly dosage between 75 and 100 milligrams but this was not always possible as several of the patients lived at some distance from the Clinic.

The most encouraging aspect of this work is the effect of the male sex hormone on the pain and disability of the patient. This was markedly demonstrated in three of our five cases, of which cases one and four are noteworthy. The former was bed-ridden because of severe leg pain due to extensive bony metastases. After the first week of therapy she became ambulatory and several weeks later felt well enough to travel. The latter patient was taking narcotics to relieve pain in her neck and shoulders which was severe enough to confine her to bed. After three weeks of therapy she was able to do without the narcotics and to get out of bed although she did not become completely ambulatory. In case five, in spite of the brief period of therapy and her terminal condition, relief of pain was sufficient to allow movement of the upper extremities the day before death. This she had been unable to do when admitted to the hospital. Although it is difficult

to evaluate, it is our impression that there was a definite improvement in appetite and sense of well being in three of the five patients. This relief from pain, especially that due to skeletal metastases, has been observed by others. Farrow and Woodward (4) mention relief from pain in about one half of thirty-three patients, although there was no evidence of control of the disease. Fels (5) described alleviation of pain in two patients. The results in one were quite dramatic, the patient becoming ambulatory after being bed-ridden for eighteen months because of pain. Adair and Herrmann (1) likewise obtained relief from pain in three patients. Boger (3) reported similar results in one patient treated with surgical castration and oral androgen.

Weight increase occurred in two of our patients, cases one and two. We found a direct correlation between the patient's weight and the administration of androgen. One maintained her weight and the other showed an increase during the course of therapy. Upon withdrawal of the androgen, both patients showed a definite progressive loss of weight. This is depicted in Figure 1. Loeser (10) reported a case in which there was improved health and a gain of five pounds. The improvement lasted four months after cessation of testosterone therapy, and then the weight began to decline. Adair and Herrmann (1) observed weight gains in three of four patients receiving large doses of testosterone. Their patients gained as much as fifteen pounds in eight weeks. Since loss of weight is a poor prognostic sign in patients with carcinoma, the weight gains observed with the use of testosterone propionate are encouraging. We recognize the possibility that the weight gain may be a direct effect of the androgen rather than a result of an inhibition of malignant processes.

The serum calcium, inorganic phosphorus, and acid and alkaline phosphatases<sup>3</sup> were followed in three of the patients. One of these, case one, had numerous skeletal metastases. She received 500 milligrams of testosterone propionate in a six weeks period. Ninety days after cessation of therapy, calcification of the osteolytic skeletal metastases was demonstrable by roentgenogram. Following this her therapy was sporadic and inadequate (she was reluctant to return to the Clinic) and the bone destruction increased. Her serum calcium during this period fluctuated between 9.3 and 15.3 milligrams per cent. At one time after receiving two 50 milligram doses of testosterone ten days apart, the calcium rose to 15.3 milligrams per cent. The subsequent determination five months later was 11.8 milligrams per cent. Serum alkaline phosphatase levels, which were determined coincidentally with the calcium, were within normal limits. These results are

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<sup>3</sup> We wish to express our appreciation to Dr. Carroll F. Shukers, Department of Biochemistry, for performing the serum phosphatase determinations.

compatible in part with those reported by Farrow and Woodward (3). They found that the serum calcium of patients with carcinoma of the breast and skeletal metastases fluctuates spontaneously. They treated thirty-three patients with a maximum total dose of 300 milligrams of testosterone, and three patients with larger doses of testosterone plus estrone. These patients showed an increased activity of the bony metastases. Adair and Herrmann (1) found improvement and calcification of osseous lesions in three of their cases, two of which had a concomitant rise in the serum alkaline phosphatase. They used massive doses of testosterone propionate, with a total dose of about 4000 milligrams. Our patient showed improvement at first on regulated therapy, and then regression when therapy became irregular and inadequate. The observations of Farrow and Woodward, Adair and Herrmann, and ourselves would suggest that small or inadequate doses of androgen tend to activate skeletal metastases, while larger doses over a relatively short period are beneficial. The two cases without skeletal involvement showed no abnormality in the serum calcium, phosphorus or phosphatases.

The effect of testosterone on the primary breast tumor and soft tissue metastases is discouraging with the dosage we have used. Case three received 550 milligrams in 105 days with no detectable changes. The ulcer became cleaner and lost much of its odor, but this could well have been due to the topical therapy administered. In case two the primary tumor definitely increased about three times in size and became more fixed to both skin and anterior chest wall. This was after a total administration of 1425 milligrams of testosterone in 105 days. Adair and Herrmann (1) had one case of almost complete regression of the primary tumor and axillary metastases with a dosage of 3975 milligrams over a 105 day period. Therefore the lack of response in our case may have been the result of too small a dosage. Two of our patients, cases one and four, had superficial soft tissue metastases; in one case to the skin of the chest wall, and in the other to the supraclavicular and cervical lymph nodes. Our treatment here also failed to elicit a favorable response. The skin metastases in case one actually increased in number although the skeletal metastases showed improvement. A very interesting phenomenon was observed in case four which we cannot explain. This patient complained on several occasions of a transient feeling of fullness and swelling in the region involved by the cervical metastases. This occurred about twelve to twenty-four hours following each of several injections of testosterone and seemed to be definitely correlated with it. Our knowledge of the effect of testosterone on tumor cells is still too incomplete to offer an explanation.

We encountered no serious toxic reactions with testosterone propionate in sesame oil (Oreton). Most of the patients had the expected vaso-dilata-

tion. Although one patient had a generalized pruritus during therapy, this subsided spontaneously without stopping treatment. We cannot definitely ascribe this condition to the effects of the hormone. Amenorrhea occurred in the one patient who was menstruating prior to treatment. This was temporary and menses occurred four weeks after the cessation of treatment. Hirsutism and flattening of the non-cancerous breast occurred in this same patient who had received 1425 milligrams of testosterone propionate. This was the only patient who showed evidence of virilism.

There were no effects of the androgen on the hemograms, urinalyses, and serum proteins in any of these patients. One patient had a leukopenia when therapy was instituted, but this remained unchanged both during and after treatment.

Our results in this study are sufficiently encouraging to us to warrant our continuation of this method of treatment of inoperable carcinoma of the breast.

### CONCLUSIONS

1. Five patients with carcinoma of the breast and metastases to the soft tissues and skeleton were treated with testosterone propionate.
2. Following therapy symptomatic relief of pain and increased sense of well being of the patients were pronounced.
3. Moderate doses of testosterone propionate have no beneficial effect on the primary tumor or soft tissue metastases.
4. The results suggest that recalcification of osteolytic metastases occurs with adequate therapy.
5. No serious side reactions were noticed with this method of treatment.

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# SYMMETRIC CEREBRAL CALCIFICATION WHICH FOLLOWED POSTOPERATIVE PARATHYROID INSUFFICIENCY: REPORT OF A CASE

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THREE communications pertaining to symmetric cerebral calcification were published by Camp, Love, Eaton and Haines (2, 3, 5) in 1938 and 1939, and a thesis on this subject was prepared by one of us (Eaton (1)). In these communications were described some of the roentgenographic (Fig. 1a and b) observations in seven cases and the data obtained at necropsy in one of these cases.

The point was emphasized that symmetric cerebral calcification was often associated with spontaneous parathyroid insufficiency, and it was our opinion that the changes in the brain resulted from parathyroid insufficiency. This hypothesis could, of course, be somewhat strengthened by the finding of symmetric cerebral calcification in a case of postoperative parathyroid insufficiency, but this we had been unable to do at the time of our last publication. Failure to find such an occurrence may have been due in part to failure in earlier years to make roentgenograms of the skull in our cases in which parathyroid insufficiency followed thyroidectomy, and in part, also, to the fact that whereas the condition of most patients who have postoperative parathyroid insufficiency is properly diagnosed and treated early, many patients with spontaneous parathyroid insufficiency are untreated for many years. It is generally true also that, in most cases, spontaneous parathyroid insufficiency is of severe degree, whereas a larger number of those patients who acquire the deficiency after operation have it to a less severe degree.

In 1939, Kahn, Lion and Zimmerman (4) reported a case in which cerebral cortical calcification simulated Piek's disease. The disease occurred in a patient who had postoperative parathyroid insufficiency, and study of tissue removed from the brain showed the same type of change that we had found in the brain of one of our patients. In this case the chronic parathyroid insufficiency appeared to have been present for fourteen years. The authors have discussed the possibility that the cerebral calcification might be due to parathyroid insufficiency.

The occurrence of calcification in patients who have parathyroid insufficiency has been further corroborated by the finding of roentgenographic

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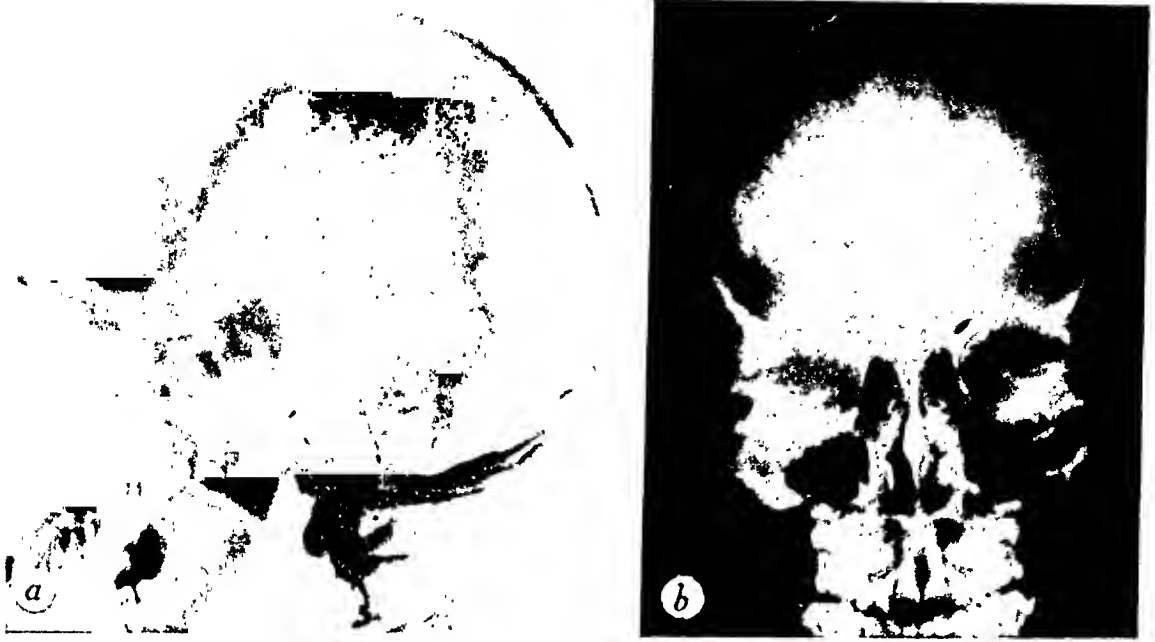


FIG. 1a. Left lateral view of the skull of a boy thirteen years and seven months old, with spontaneous parathyroid insufficiency showing areas of calcification below the usual situation of the anterior horn; *b*, anteroposterior view of the skull of the same patient, showing symmetric areas of calcification in the region of the basal ganglia.



FIG. 2a. Left lateral aspect of the skull of the patient in the case reported in this paper, showing extensive calcification of the basal ganglia, and slight calcification of the cerebral hemisphere above the basal ganglia; *b*, anteroposterior view of the skull of the same patient, showing symmetric distribution of the calcification.

evidence of cerebral calcification in 11 of 17 patients with spontaneous parathyroid insufficiency studied at the Mayo Clinic from 1935 through 1945 and by the finding, in one of these 17 cases, of calcification in an area of the subcutaneous tissue.

The present report deals with a patient who had postoperative parathyroid insufficiency and who also had symmetric cerebral calcification.

### REPORT OF A CASE

A factory worker forty-two years old entered the Mayo Clinic on January 1, 1944, complaining of poor vision and frontal headache. Eighteen months prior to admission, he first noted difficulty with his vision. New glasses had been fitted; they had maintained improved vision for about a year. At the end of that period blurring of vision had become progressively worse.

When we saw the patient he had been suffering for about two months from persistent dull frontal headache and occasional sharp, shooting pains over both temples. There were also sharp frontal pains which might occur at any time of the day or night. Pains in the head did not become worse during coughing, sneezing or bending, but diplopia occurred frequently. An anodyne (anacin) gave relief. The patient's wife stated that he was becoming much more irritable, fidgety and childlike, and that his memory had deteriorated markedly. The possibility of his having a brain tumor had been considered.

The history of this patient revealed a chronically nervous disposition that had become worse during the previous ten years. He had had a goiter and had complained of dyspnea in 1920. Thyroidectomy had been performed. On the morning after thyroidectomy he had experienced carpopedal spasm, with recurrent attacks at three months and twelve months postoperatively. The patient then had been free from such attacks for eighteen years, although he said he occasionally felt "dizzy and lightheaded."

In 1940, he had experienced a fourth attack of carpopedal spasm, followed by several attacks which had occurred about every other day. One episode had awakened him from sleep, and he had noted that he usually slept for several hours after an attack. He had never bitten his tongue or become incontinent during an attack. There was no family history of convulsions. His only aura consisted of a sensation of dizziness before attacks; he rejected the possibility of hyperventilation. The last episode of carpopedal spasm had occurred in April, 1943, about eight months before the patient came to the clinic. The patient drank much milk, and stated that he thought food relieved his nervousness.

Examination disclosed a well-developed, well-nourished white man who exhibited an occasional muscle tremor. Blood pressure was 115 systolic, and 70 diastolic, expressed in millimeters of mercury. The pulse rate was 66. Residual thyroid tissue, palpable on the left, was estimated to be about 2.5 by 5 cm. A presystolic murmur could be heard at the apex cordis, and there was a diastolic murmur at the pulmonie area. Reflexes were moderately diminished bilaterally. Chvostek's sign was elicited, and Trousseau's sign was pronounced. The ocular fundi appeared to be normal, so far as they could be seen, but diffuse opacity of the lenses of both eyes was noted. Results of the urinalysis, of the Kline test of the serum, and of roentgenograms of the thorax were negative. Results of blood counts and of electrocardiography were within normal limits. The basal metabolic rate was -4 per cent. Roentgenograms of the skull were reported as demonstrating "very extensive calcification of the basal ganglia on both sides, especially on the right. There is some slight calcification in both cerebral hemispheres above the basal ganglia" (Fig. 2a and b).

The value for serum calcium was 5.5, and that for phosphorus, 6.6 mg., per 100 cc. of serum. Two grams of calcium lactate in solution was administered in doses of one teaspoonful (about 4 cc.) four times daily, along with two capsules (1.25 mg.) of dihydrotachysterol, for two days; then one capsule (0.625 mg.) of dihydrotachysterol was administered daily. Cataract surgery was deferred for several months.

The patient returned to the clinic on March 12, 1945, for removal of cataracts. His sight had become progressively worse, so that he had been unable to work, but he was much less nervous than formerly, and had not had convulsions or numbness and tingling of the hands since the institution of adequate therapy. He had continued to take one teaspoonful of calcium lactate three times daily, but had not continued to take dihydrotachysterol. He had gained 5 pounds (about 2 kg.) during the two months prior to this second visit to the clinic.

Results of examination were as previously noted except that Chvostek's and Trousseau's signs were not obtained. Studies of serum chemistry at this time showed the value for calcium to be 9.0, and that for phosphorus to be 5.4 mg., per 100 cc. of serum. The patient underwent operation on March 19, 1945, at which time an immature cataract was removed from the right eye. Recovery was without incident.

### COMMENT

This case, illustrating the occurrence of symmetric cerebral calcification in a patient who had parathyroid insufficiency which apparently developed after thyroidectomy, seems to us to support the opinion that the characteristic cerebral pathologic process actually is secondary to parathyroid insufficiency. The intermittency of symptoms in this instance is similar to that seen frequently in cases of postoperative parathyroid insufficiency. The control of symptoms, including convulsions, without demonstrable changes in the roentgenologic picture of cerebral calcification, is also similar to the observation in other patients. In fact, in one of our patients (Fig. 1*a* and *b*) with spontaneous parathyroid insufficiency—a boy thirteen years and seven months old who had had convulsions for six years—continuous treatment resulted in control of convulsions and was accompanied by a rise in the intelligence quotient from 73 to 99, although the cerebral calcification showed no change over a period of nine years. Similar experiences have been noted in other patients.

### SUMMARY AND CONCLUSIONS

Symmetric cerebral calcification, demonstrable roentgenographically, frequently is observed in the presence of idiopathic parathyroid insufficiency, and is herein reported in a case in which parathyroid insufficiency followed thyroidectomy. This case supports the opinion that the cerebral changes are secondary to the metabolic disturbance resulting from insufficient parathyroid secretion, and that these cerebral changes are not the cause of the disturbance. Adequate treatment of parathyroid insufficiency leads to relief of clinical symptoms, but does not cause the cerebral calcification to disappear.

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# FEMINIZING TUMOR OF THE TESTIS

## PRESUMABLY ABERRANT ADRENOCORTICAL TUMOR

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**I**N MOST instances of feminizing tumors observed in adult males, the most conspicuous symptom of feminization is gynecomastia, accompanied by more or less pronounced genital atrophy and sometimes but not always, loss of capacity for sexual function. The appearance of gynecomastia may also be due to other causes, however. Thus the phenomenon has been described as a hereditary familial character (5). In lesions producing atrophy of the testis, or after castration, gynecomastia or gynecomastia-like deposits of fat may appear even though this is far from being the rule (15).

The tumors which in adult males have given rise to feminization have originated from the adrenal cortex or testis. The feminizing adrenocortical tumors have most often been carcinomas, but on the whole they are rare. In 1942 Roholm & Teilum (17) described such a case, reviewing at the same time the 6 instances of feminizing adrenocortical tumors previously reported.

When adrenocortical tumors are able to alter the sex characteristics usually in the male direction, the change is due to production or, rather, hyperproduction of androgens or estrogens respectively. Whenever testicular tumors have been feminizing, physical changes have also been due to hormonal production. At any rate this applies to the highly malignant chorionepithelioma of the testis—the testicular tumor which most often gives rise to gynecomastia. Aside from chorionepithelioma, gynecomastia has been observed in a few cases of other malignant testicular tumors—as in seminoma (21, 8) and embryonal carcinoma (8). In such cases of malignant testicular tumor it is difficult to establish the genesis of the gynecomastia, which perhaps may be due to the prevention of the testicular function by the tumor rather than to any endocrine function of the tumor itself.

Finally, gynecomastia has been observed a few times in cases of non-malignant testicular tumors; in two cases of interstitial cell tumor (14, 10) and in one case of testicular paraganglioma. These cases will be mentioned further in the discussion of the following instance of feminizing testicular tumor observed by the writer.

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\* Surgeon-in-chief: C. D. Bartels.

### CASE HISTORY

The patient was a farmhand, 28 years old, who was admitted to the hospital in March 1942. In the last couple of years he had noticed that the mammae were increasing in size and he entered the hospital to be treated for this anomaly.

There had been no pain or unusual sensation in the mammae, nor any secretion. He does not remember anything about previous traumatic injury to, or pain in the scrotal region, and he had not noticed any enlargement of the left testis. Libido and sexual potency were said to be normal.

He was of medium height, of virile build with well-developed muscles and no excess



FIG. 1

adipose tissue. His hairiness was of the masculine type. The mammae were rounded as in a young girl, with distinctly well-developed mammary glands. No secretion could be expressed from the papilla (Fig. 1). An examination of the genitals showed the pubes to be normal and the penis normally developed. Both testes were in the scrotum but the right was somewhat atrophic, about the size of a pigeon egg. The left testis was about twice as large, and at its upper pole a well-defined tumor was palpable.

Surgical intervention by semicastration was performed on March 20. The removed testis contained a well-defined tumor, about the size of a large hazel-nut, arising from the testicular hilus; on section, the cut surface was yellowish-orange in color.

*Histological Description* (Dr. Fr. Gregersen): The testis showed the presence of a roundish tumor that had no connection with the tubules and no relation whatever to the testicular parenchyma, being surrounded by a capsule of connective tissue. The tumor was lobated by rather coarse streaks of connective tissue; it was very rich in cells, being



made up of rather large cells, arranged in alveolar heaps or in narrow streaks, here and there in rosette-like pattern, surrounded by capillaries and sinusoids, and in some areas by delicate connective tissue septa. The cells were rather rich in protoplasm, varying in form from plump spindle-formed to more cylindrical, somewhat epithelioid cells, often adjusted in form to each other. In sections stained after v. Giesen-Hansen the cells were smoky-yellowish in color. The nuclei were rather large, round or slightly ovoid, with a single nucleolus. The tumor tissue had nothing in common with tumors arising from testicular parenchyma, and there was nothing suggestive of ovarian tissue. As the specimen was fixed in formalin-alcohol, it was not possible to judge whether it might have been chromaffin. An attempt at impregnation with silver showed the cytoplasm to be

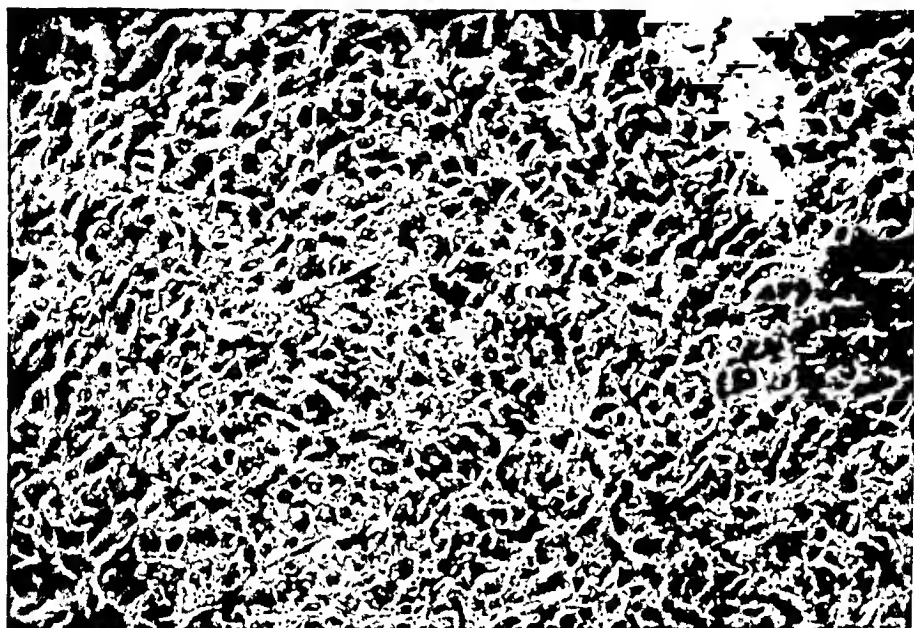


FIG. 2

coarsely granular, but there was no definite argyrophilia of the granules. Nor did the Giemsa stain give any absolutely certain color reaction, as the cells became bluish-green, not markedly green. Mitotic figures were rather numerous. According to the structure of the tumor, and the character and arrangement of the cells, it resembled a tumor arising from chromaffin tissue. Owing to the fixation of the specimen in formalin-alcohol, however, it was not possible to establish this diagnosis with certainty.

The tumor was well-defined and showed no destructive growth, so that it afforded no histological evidence of the malignancy which might have been suspected because of the unusually large number of cells and many mitoses. (Fig. 2).

Hormonal Analysis (Dr. Christian Hamburger), on March 12 (prior to the removal of the tumor): Friedman reaction negative.

Gonadotropin: 0 (*i.e.*, <50 m.u. per day).

Estrogens: about 200 m.u. per day.

Androgens: 25 i.u. per day.

On April 7 (after removal of the tumor):

Gonadotropin: about 50 m.u. per day.

Estrogens: about 50 m.u. per day.

Androgens: about 62 i.u. per day.

At the time of the patient's discharge from the hospital, about 3 weeks after the operation, the breasts had not diminished distinctly in size. The patient has been re-examined several times, most recently in April 1944. During this period the breasts have decreased gradually until they are now only slightly enlarged. Remnants of the previously enlarged mammary glands are still palpable. The patient is very satisfied with the result. The right testis has grown distinctly, being now of normal size and consistency. There has been no disturbance in sexual potency. In the meantime the patient has married and is now the father of a child. He feels perfectly well. There are no signs of metastasis from the removed testicular tumor.

### DISCUSSION

There can be no doubt that the testicular tumor in this case was feminizing. This is indicated by the fact that the gynecomastia appeared together with the development of the tumor and disappeared after the removal of the tumor. This is further indicated by the demonstration of increased estrin excretion prior to the removal of the tumor and normal estrin excretion after the operation, as well as by the previous atrophy of the right testis and its subsequent growth after the operation. There was no disturbance of the sexual potency, it is true, but this feature was also absent in several of the cases mentioned in the literature.

In trying to classify the present tumor among the feminizing testicular tumors mentioned in the introduction, the possibility of chorionepithelioma can be excluded at once because of the morphology of the tumor, the course of the case and the absence of excretion of chorionic gonadotropin with the urine. Nor has this tumor anything in common with seminoma or embryonal carcinoma. On the other hand, the tumors arising from interstitial cells in the testis are rather to be taken into consideration. According to the prevailing view, the interstitial cells (Leydig's cells) in the testis produce a masculine hormone. In keeping with this view pubertas praecox has been observed in the cases of boys in whom interstitial cell tumors have been described (19, 22). In those cases—nearly 20—in which interstitial cell tumors have been described in adult males, changes in the sexual characteristics were observed only in the two reported by Monaschkin (14) and Hunt and Budd (10), where the tumor was accompanied by development of gynecomastia and, in the case of Hunt and Budd, also with disturbances in sexual potency.

The interpretation of these two cases, however, is open to criticism. Thus Mathias (9) thinks that in Monaschkin's case the lesion involves an inflammatory reaction resulting in an increase in interstitial cells, not any true tumor formation. This patient presented a distinct atrophy of the other testis, aspermia and feminine limitation of the pubes, on which account Mathias thinks that the patient was a castrate-like, slightly feminine

male with gynecomastia that could not have been produced by increase in interstitial cells.

In Hunt and Budd's patient, a man of 42 years, the feminizing testicular tumor was well-defined, 2–2.5 cm. in diameter, situated in the mediastinum of the testis, yellowish on the cut surface, and morphologically the tumor cells resembled interstitial cells. The urine showed an excretion of 1000 units of luteinizing gonadotropin (chorionic gonadotropin?) per liter. The excretion of estrogen and androgen was not examined. The increased excretion of luteinizing gonadotropin is difficult to understand in connection with an interstitial cell tumor; it rather suggests that the tumor or the removed testis contained chorionic elements. But this assumption is not corroborated by the course of the case, as no recurrence or metastasis was observed during a period of two years. Thus it seems rather doubtful that these two feminizing testicular tumors really were interstitial cell tumors.

In its course, as well as in the size, location and macroscopic appearance of the tumor, our case resembles the case reported by Hunt and Budd. Microscopically, however, the tumor cells in our case differ somewhat from interstitial cells, being darker in color, deficient in protoplasm and more closely packed. So this could hardly have been an instance of interstitial cell tumor.

From the histological description it is evident that in its structure and cellular morphology this tumor is like those arising from the chromaffin tissue in the adrenal medulla or from the paraganglia. In the literature, an instance of gynecomastia has been described in connection with a testicular tumor reported by Botteselle as a paraganglioma.

It seems rather strange, however, and it has not been described before, that a tumor consisting of adrenomedullary cells should be able to alter the sex characteristics whereas this phenomenon has been reported not infrequently in adrenocortical tumors. So the question is if it might not be more likely in our case (and perhaps in that of Botteselle too) that the tumor was an aberrant adrenocortical one rather than a paraganglioma of adrenomedullary cells. At any rate in our case it was not possible to prove that the cells belonged to the chromaffin system, and from the only description of Botteselle's case accessible to the writer it is not evident that the tumor described as a paraganglioma was chromaffin. In these cases it would hardly be possible from the cellular morphology alone to decide with certainty whether the tumor was made up of adrenocortical or adrenomedullary cells; and it happens but seldom that an adrenocortical tumor presents the three zones of cells typical of the adrenal cortex. Morphologically, the cells in our case may very well be adrenocortical cells. From the description and the microphoto it will be noticed that in our case

the cells are arranged in alveolar heaps, narrow streaks, sometimes forming rosettes, surrounding capillaries and sinusoids, or surrounded by such vessels—a microscopic picture that corresponds very well to the features encountered in tumors of the adrenal cortex. Also the increased output of estrin and the lack of gonadotropin excretion correspond to findings that may be expected in a feminizing tumor of the adrenal cortex.

It may perhaps seem rather surprising that a tumor of the testis should consist of adrenocortical cells. Still, from the pathological anatomy we know that adrenocortical tissue may occur in other parts of the organism besides the adrenal. Small accessory adrenals have been demonstrated not only in the immediate vicinity of the adrenal but also along the course of the internal spermatic vein (2) and in the ligamentum latum, near its lateral margin, where the occurrence of these bodies was first demonstrated by Marchand (11), on which account they often are designated as adrenals of Marchand. Marchand (1882) described then a small yellowish body, 1–3 mm. in diameter, built up histologically as adrenocortical tissue. Later, such bodies have been demonstrated also between the testis and epididymis (3), in the mediastinum of the testis (18), in the vicinity of the epididymis (23) and the ductus deferens (20, 13). They are found fairly often in the newborn and infants, but have been demonstrated also in adults. As a rule, they are quite small, only a few millimeters in diameter but in rare instances they have attained cherry size. In certain cases these accessory adrenals have been looked upon as the origin of tumor formation (16).

In a very few cases, changes in the sexual characteristics have been described in connection with such aberrant adrenocortical tumors located in the ligamentum latum (12, 7, 6, 4). As far as the writer has been able to find out, hitherto no instance has been described of a feminizing adrenocortical tumor localized to the testis. As mentioned, however, several things suggest that the tumor here described constitutes such a case, perhaps developing from a Marchand adrenal located in the hilus of the testis.

Nearly all the feminizing adrenocortical tumors reported previously (17) have been malignant. On account of its richness in cells and the rather numerous mitoses, the tumor here described is a little suspect of malignancy. Possibly these characters of the present tumor are the reason why it produced estrogen and brought about the change in the sexual characteristics in contrast to most other aberrant adrenals or instances of hyperplasia or tumor formation developing from such bodies. In the present case, however, there was no definite histological evidence of malignancy of the tumor and the course of the case under two years observation without any recurrence or metastasis, excludes the probability of malignancy.

## SUMMARY

Reference is made to the various feminizing tumors, and an account is given of their mode of action.

The feminizing testicular tumor observed by the writer was found in a man of 28, in whom gynecomastia had developed in a couple of years. In addition there was atrophy of the non-tumor-bearing testis, increased excretion of estrogen with the urine, but no output of gonadotropin and no disturbance of sexual potency. After removal of the tumor, the estrogen output decreased to normal, the gynecomastia subsided almost completely within two years, and the other testis increased to normal size. The tumor removed was a little larger than a hazel-nut, well-defined, localized to the hilus of the testis. On the section surface it was yellowish-orange in color. Histologically it showed no resemblance to any of the tumors usually occurring in the testis.

As to the nature of the tumor, the author discusses the more obvious possibilities of interstitial-cell tumor, paraganglioma and adrenal cortical tumor. On the basis of the histological features and the endocrine function of the tumor, the author arrived at the conclusion that presumably it was an aberrant adrenal cortical tumor, developed from accessory adrenal cortical tissue located in the hilus of the testis.

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THE ASSOCIATION  
FOR THE STUDY OF  
INTERNAL SECRETIONS  
TWENTY-NINTH ANNUAL MEETING

FRIDAY AND SATURDAY, JUNE 6-7, 1947

*Haddon Hall, Atlantic City, New Jersey*

ABSTRACTS OF PAPERS PRESENTED  
ABSTRACTS ARRANGED ACCORDING TO NUMBER

Friday, June 6

9:30 A.M. *Viking room*

1. STUDIES IN CORPUS LUTEUM FUNCTION.

J. S. L. Browne, J. S. Henry and E. H. Venning. (From the McGill University Clinic, Royal Victoria Hospital, Montreal, Canada, and the Department of Obstetrics and Gynecology, McGill University.)

Sodium pregnanediol glucuronidate was determined by the method of Venning and correlated with endometrial biopsies. In 21 cycles in 20 cases biopsies showed no progestational changes when taken just before the onset of the menstrual period, and, the pregnanediol in the same cycle was absent. In 45 cycles in 36 cases the biopsies showed early or late progestational changes and this was well correlated with the time of onset or the presence of pregnanediol. On no occasion was the pregnanediol positive in a cycle in which the biopsy showed no progestational change. There were no cases of adrenal tumours with amenorrhoea in this series; in such cases pregnanediol has been found to be positive with an atrophic endometrium. On 10 cycles in 8 cases the biopsies showed progestational changes but no pregnanediol was present in the same cycle. Some of these discrepancies are explicable on the grounds of very large volumes of urine which prevented the detection of pregnanediol. The effect of chorionic gonadotropin on pregnanediol excretion has been further studied. If chorionic gonadotropin is administered in a dose of 1000 international units daily in the early part of the luteal phase, in some instances a marked increase in the excretion of estrogen occurs (up to as high as 3,800 units in one case). The excretion of pregnanediol is prolonged and endometrial biopsies show histological changes similar to those seen in early pregnancy. In certain cases however, the corpus luteum function cannot be prolonged by the administration of this dose.

2. PROGESTERONE THERAPY OF UTERINE FIBROMYOMATA.

Albert Segaloff, John C. Weed (by invitation) and William Parson. From The Alton Ochsner Medical Foundation, The Ochsner Clinic and the Departments of Medicine and Gynecology, Tulane University of Louisiana School of Medicine, New Orleans, Louisiana.

Patients with uterine fibromyomata have been treated with progesterone.

Only those patients in whom it has been possible to outline the tumor by roentgenography with either intraperitoneal carbon dioxide or the intrauterine instillation of radiopaque oil have been followed.

Three patients were treated for 39 to 61 days with daily intramuscular injections of 20 mg. of progesterone. One patient was given 100 mg. of anhydrohydroxy progesterone by mouth daily for eight weeks.

All four of these patients have come to surgery. There was no decrease in the size of the fibromyomata. Many of the fibromyomata showed microscopic evidence of regression.

Three additional patients have been implanted with 7 or 8 200 mg. pellets of progesterone. They have been followed from one to six months but none have as yet come to surgery.

There has been no significant interference with the menstrual cycles. Urinary gonad stimulating hormone and 17-ketosteroid excretion have also not deviated significantly from the pretreatment values.

### 3. DISTORTION OF THE SPIRAL ARTERY IN THE OVARY IN THE PRESENCE OF CORPUS HEMORRHAGICUM CYSTS AFTER ADMINISTRATION OF GONADOTROPINS TO RABBITS.

S. R. M. Reynolds, Carnegie Institute of Washington, Department of Embryology, Baltimore 5, Md.

The terminal arterial supply of the ovary arises from the coils of one or two spiral arteries lying along the hilus of the ovary. This arrangement, it has been surmised, lowers arterial blood pressure within the ovary to near-osmotic levels, and equalizes it throughout the ovary. After injection of gonadotropins, the coils become "paid out" evenly during the initial response of ovulation, luteinization, and generalized follicular growth. Accordingly, the spiral arrangement of the main arterial supply to the ovary permits ready adjustment to an increase in length of the ovary.

In a series of rabbits receiving gonadotropins, and sacrificed at twenty-four hour intervals, a certain number showed fresh corpora hemorrhagica. Within the first twenty-four hours, the coils were evenly extended regardless of whether or not fresh small corpora hemorrhagica were present. It was observed that whenever large corpus hemorrhagicum cysts (measuring from about 2 to 3 millimeters across) were present two to six days after injection the cysts were distal to a region of disruption in the orderliness of the coils. Recent cysts have been seen on the ninth day which were associated with symmetrically arranged spiral arteries as well as with ones which were disrupted.

All specimens were injection-corrosion preparations in which vynilite was used as the injection mass. The preparations were studied from stereoscopic photographs enlarged two or three times.

### 4. GONADAL STIMULATION FOLLOWING THE ADMINISTRATION OF ANTIGONADOTROPIC SERUM.

Herbert S. Kupperman, Roland K. Meyer and J. C. Finerty. From the Zoological Laboratories, University of Wisconsin, Madison, Wisconsin, and the University of Georgia School of Medicine, Augusta, Georgia.

Antigonadotropic serum obtained from rabbits injected with aqueous (unfractionated) extracts of sheep pituitary was administered to immature rats. The serum was injected for a period of 10 days to two groups of rats, one and ten days old, respectively. Vaginal introitus occurred in the animals treated with antigonadotropic serum 3-4 weeks prior to that in the littermate control. Patency of the vagina occurred, on an average, 10.9 days after cessation of antigonadotropic therapy. Associated with the observed precocious sexual maturity there was marked ovarian and uterine stimulation over and above that seen in the control females. Basophilic changes were observed in the pituitary



glands after the ten day treatment with antihormone. However, gonadotropic assays performed at this time showed the pituitary of the antihormone-treated rats to contain less gonad-stimulating substances than that of littermate controls. These data demonstrate that precocious sexual maturity, endogenous in origin, may be the result of morphological changes induced in the hypophysis of the injected animals. The data present evidence that the ovary of the sexually immature rat secretes a gonadal hormone, the absence of which will cause basophilism in the anterior pituitary.

5. LIVER AND GONADAL CHANGES FOLLOWING THE ADMINISTRATION OF CARBON TETRACHLORIDE TO MALE RATS AND FEMALE GUINEA PIGS.

Boris Krichesky, S. J. Glass, E. Furlong and M. Feiner, Department of Zoology, U.C.L.A.

Previous experimental and clinical observations suggest:

1. That a liver-gonadal syndrome may follow or be associated with sustained and extensive liver damage,
2. That the impairment of gonadal function may be the end result of hepatic failure to inactivate the steroid hormones, especially the estrogens and androgens.

To test this theory, further observations on this liver-gonadal relationship are being reported. It was found that the feeding of carbon tetrachloride to male rats and female guinea pigs was followed by hepatic cirrhosis and gonadal damage in both groups of animals. In the guinea pigs, estrogen excretion is strikingly increased for over 50 days to be followed by a drop within 60 days to pretreatment levels. The ovaries at the terminal phase of this experiment show striking atrophy.

Whether these gonadal effects are a direct result of the liver damage and/or changes in pituitary gonadotropic function is being investigated.

Lantern slides of the various morphologic changes observed in the sequence of progressive liver and sex organ damage will be shown.

6. CLINICAL EVALUATION OF DIENESTROL, A SYNTHETIC ESTROGEN.

A. E. Rakoff, K. E. Paschkis and A. Cantarow. Jefferson Medical College and Hospital.

Dienestrol (4:4' dihydroxy- $\gamma$ - $\sigma$ -diphenyl- $\beta$ : $\sigma$ -hexadi-) was evaluated in 82 patients with various conditions in which estrogen therapy was indicated with reference to clinical improvement, objective response, and evidences of toxicity.

In 40 menopausal women dienestrol afforded excellent relief in every instance, the minimal dosage necessary ranging from 0.1 to 1.0 mg. with an average of 0.43 mg. daily. However, the average dosage required to improve the deficiency smear to a slight estrogen effect was 0.77 mg. while to produce a marked estrogen effect required much higher dosages. Bleeding during the course of treatment or on withdrawal occurred only twice on therapeutic dosages and in two other cases with higher dosages. It was difficult to induce bleeding in younger women with amenorrhea even with high dosages. In 26 postpartum patients inhibition of lactation was readily obtained with dosages of 1.0 mg. daily for 3 days and 0.5 mg. thereafter for 1 week. These findings suggest that dienestrol has a relatively marked inhibiting effect on the pituitary.

No symptoms of intolerance to dienestrol were noted in any patient even in high dosage. In 25 patients treated for 4 to 12 months (average 6½ months) no evidences of toxicity were noted nor any significant changes in blood count, urinalysis, or liver function studies.

7. DIETHYLSTILBESTROL DIPALMITATE IN AQUEOUS SUSPENSION.

S. Charles Freed, Department of Medicine, Mt. Zion Hospital, San Francisco, California.

We have demonstrated that there are at least two advantages in preparation of fat soluble crystalline sex hormones suspended in aqueous medium (estrone, stilbestrol and testosterone). These advantages are freedom from the allergic reactions of the usual oil vehicle and increased therapeutic effectiveness through a delay in absorption of the aqueous suspension over that of the oil solutions. Diethylstilbestrol dipalmitate, a slowly absorbed estrogen with prolonged action was suspended in an aqueous medium to determine whether this possessed the advantages over an oil solution. Five mg. of the material containing 1.6 Gm. of the active component was administered every two weeks to menopausal patients according to the plan established for the other preparations. Eighty-six patients received 242 treatments. There appeared to be no appreciable difference in the beneficial effect of these patients with a comparable number of patients receiving an equal amount of the same material dissolved in oil. Either the oil solution is as slowly absorbed as the aqueous suspension or else there is no enhancement of effect by further slowing down the absorption of this preparation. The only advantage therefore of an aqueous suspension of diethylstilbestrol dipalmitate over that in oil is its freedom from local allergic reactions.

#### 8. THE EFFECT OF DIETHYLSTILBESTROL UPON ALLOXAN DIABETES AS RELATED TO FOOD INTAKE IN THE RAT.

Dwight J. Ingle. The Research Laboratories, The Upjohn Company, Kalamazoo.

Diethylstilbestrol and other estrogens were found to be diabetogenic in the partially depancreatized, force-fed rat (Ingle, D. J., *Endocrinology* 29: S38, 1941), whereas Janes and Dawson (*Endocrinology* 38: 10, 1946) failed to observe a diabetogenic effect of diethylstilbestrol in alloxan-diabetic rats which ate *ad libitum*.

The present experiments show that the effect of diethylstilbestrol upon alloxan diabetes is determined by the intake of food. Male rats of the Sprague-Dawley strain (initial weight, 300 grams) were made diabetic by repeated intraperitoneal injections of 25 mg. of alloxan. All of the animals were fed a fluid medium carbohydrate diet.

When diethylstilbestrol was administered to animals in which the food intake was kept constant by forced feeding, there was a striking exacerbation of the diabetes in every case, and the effect disappeared when the administration of the estrogen was stopped.

Those animals which ate *ad libitum* voluntarily consumed more food than was given by feeding, but when the estrogen was administered the food intake dropped sharply and the amount of urinary glucose was decreased significantly.

#### 9. THE FACTOR OF PREVIOUS TREATMENT IN EXPERIMENTAL MENSTRUATION.

Doris H. Phelps. Department of Obstetrics and Gynecology, Vanderbilt University School of Medicine, Nashville, Tennessee.

It has been shown previously that the duration of experimentally induced menstruation can be influenced by a number of factors; among these are the intensity of hormonal stimulus, its composition and the duration of its action. This report deals with a hitherto undescribed factor of great importance, namely, the structure of the endometrial vascular bed at the beginning of the cycle.

To obtain information concerning mechanisms involved in the production of menstrual abnormalities, eight ovariectomized monkeys were injected with various combinations of the ovarian hormones. Endometrial vascular phenomena were observed by means of intraocular endometrial transplants and these observations were correlated with observations on uterine bleeding. One hundred seven cycles of uterine bleeding were produced, 1-13 per monkey. Analysis of the results of each experiment in relation to treatment administered in previous experiments in the same animal revealed that 1)

permanent changes in the structure of the endometrial vascular bed may result from stimulation by the ovarian hormones and 2) the architecture of the endometrial vascular bed at the beginning of any given course of treatment with ovarian hormones exerts an important influence upon the uterine bleeding produced by that course of treatment.

#### 10. EXPERIMENTAL ALTERATION OF THE HUMAN OVARIAN CYCLE BY ESTROGEN.

Willis E. Brown, J. T. Brandbury, A. F. Jennings. University of Iowa, Department of Obstetrics and Gynecology, Iowa City, Iowa.

Patients were treated with stilbestrol at different phases of the cycle to determine its effect on menstrual rhythm and endometrial histology. When a single 20 mg. dose of stilbestrol was given orally in the early proliferative phase (4th to 10th day), the length of the interval was increased to an average of 38 days (range 32-48 days in 14 trials). Twelve untreated cycles in these patients averaged 29 days in length (range 24-35 days). Six of the treated cases experienced a very scant vaginal bleeding for a few days starting about a week after receiving the estrogen. The endometrium remained in a proliferative phase until about 10 days before the onset of bleeding and then it exhibited the normal secretory changes. There was one exception, a cycle of 33 days, in which the endometrium did not exhibit any secretory changes, suggesting an anovulatory cycle. Under these conditions, a single large dose early in the cycle seems to delay ovulation about nine days.

Eight patients with dysmenorrhea were treated with oral stilbestrol starting the day after the cessation of menstruation. The dosage was 1 mg. daily for 10 days, 2 mg. daily for the second 10 days, and 3 mg. daily for the third 10 days. With this regimen the patients were relieved of their dysmenorrhea for three successive months and endometrial biopsies indicated that all three cycles had been anovulatory. Two other patients became so nauseated that they discontinued the treatment. One patient experienced a marked increase in weight, due to an edema which subsided quickly after stopping the medication. She then had an interval of 55 days before her next spontaneous menstrual period. Under these conditions, a continuous and increasing dosage for three weeks, it is possible to suppress the luteal phase for as long as three successive months.

When estrogen medication was started in the post-ovulatory phase of the cycle, 5 to 10 mg. of stilbestrol per day, there was no appreciable alteration of the luteal phase or any delay in the onset of menstruation. To date it has not been possible to prolong the life of the corpus luteum by estrogen therapy in the woman.

#### *Papers read by title*

#### 11. STUDIES ON THE VARIATIONS OF BLOOD GONADOTROPINS AND VAGINAL SMEARS DURING PREGNANCY IN CORRELATION WITH THE FETAL SEX.

H. E. Nieburgs, H. S. Kupperman and R. B. Greenblatt, Department of Endocrinology, University of Georgia, School of Medicine, Augusta, Ga.

The investigation of vaginal smears in 253 women during the 8th to 36th week of pregnancy revealed a progressive proliferation of the vaginal epithelium due to increased estrogen activity. This is accompanied by increased glycogen deposition and massive desquamation of cells due to the action of progesterone. In addition to these progressive changes in the vaginal mucosa a number of specific smear types were observed. These were grouped into the mucoid-cornified, glycolytic and plasmolytic smears. There was no correlation of these smears with threatened abortions or toxemias. Where specific smear types were present the sex of the infant was predetermined with an accuracy of 85.4 per cent.

In a further group of pregnant women assays of blood gonadotropins were carried out on 21-24 day old mice, estimating the FSH and LH fraction. The amount of FSH present was mainly constant, somewhat decreasing towards the later stages of pregnancy. (The determinations were made for dilution of serum up to 1:40.) With the method employed the effect of LH was either totally absent or was present in dilutions up to 1:10 and rarely in dilutions of 1:20.

Data is presented on the relation of blood gonadotropin levels to the sex of the fetus.

## 12. ORAL ESTROGEN THERAPY DURING MENOPAUSE.

Abbey David Seley, Deborah Baumgold and Samuel Vernick. Gynecological Endocrine Department, Hospital for Joint Diseases, New York City.

Management of the menopausal state by oral estrogens alone was begun on July 1, 1943 in the Gynecological Endocrine clinic of the Hospital for Joint Diseases. For the past three and one half years not a single injection of any estrogenic substance has been given. Since oral estrogens are derived from various sources and/or have different chemical structures, a comparative clinical study of 90 unselected and consecutive menopause patients was initiated in order to evaluate the effectiveness of these substances. The purpose of this report is to indicate the relative value of the various estrogenic substances used in regard to: (1) ability to control the neurocirculatory disturbance characteristic of the menopause; (2) the rate of change of the vaginal smear; (3) the possibility of carcinogenic alteration when estrogens are used in doses sufficient to control the menopause as evidenced by endometrial biopsy and biopsy or curettage of the cervical stump; (4) the number and type of induced side reactions including bleeding; (5) the final result obtained by the oral route alone a) in the average case and b) in cases complicated by menopausal arthralgia, a number of which are found in this particular hospital.

## 13. CORRELATION OF BASAL BODY TEMPERATURE CURVES WITH ENDOMETRIAL BIOPSY.

A. R. Abarbanel, Department of Obstetrics and Gynecology, College of Medical Evangelists, Los Angeles, California.

Correlation studies of several hundred endometrial biopsies with the basal body temperature curve for that cycle reveals that on the whole, the classically sharply defined diphasic B.B.T. curve is usually associated with a presumed ovulatory cycle as witnessed by successful conception or a fully developed secretory endometrium.

On the other hand, study of various aberrations revealed a secretory endometrium, especially of the so-called mixed type or immature progestational phase (day 19-22 of a 28 day cycle) when taken of the first day of menses. B.B.T. curves were either irregular or flat.

It is postulated that an immature progestational phase or a mixed type of endometrium does not necessarily represent ovulation but merely various degrees of theca luteinization without concomitant ovulation. In brief, a staircase phenomenon is postulated for the human, similar to that proposed for the macacus rhesus by Hartman.

2:00 P.M. *Viking Room*

## 14. A RAPID METHOD FOR THE DETERMINATION OF URINARY "17-KETOSTEROIDS."

I. J. Drechter, Sidney Pearson and Thomas H. McGavack, New York Medical College, Metropolitan Hospital Research Unit, Welfare Island, New York.

A method for the rapid clinical determination of urinary "17-ketosteroids" in volumes of urine as small as 10 ml. is described. Easily available, inexpensive laboratory equipment is used, and the hydrolysis extraction and assay can be completed within four hours.

Optimum conditions for the hydrolysis and extraction of urine volumes from 10 ml. to 50 are given. Heating at 80° for 10 minutes gives the apparent maximum Zimmerman color producing substances.

The results of extraction with several solvents are given. Ethyl ether gives the best results. Recoveries are good. Normal value of 38 young men between ages of 18-30 by this method range from 10 to 30 with an average of 16.0 mg. excretion in 24 hours.

#### 15. FURTHER STUDIES ON THE METABOLISM OF THERAPEUTIC DOSES OF THE NATURAL ESTROGENS IN HUMAN SUBJECTS.

Benjamin F. Stimmiel and Clair L. Stealy, Rees-Stealy Medical Research Fund, Ltd., San Diego, Calif.

We have shown that our procedure for the chromatographic fractionation and photometric estimation of urinary estrogens can be used to study the metabolism of therapeutic doses (1-2 mg.) of the natural estrogens in human subjects. These studies were made on (a) a normal human female, (b) a bilaterally ovariectomized, hysterectomized human female, and (c) a bilaterally ovariectomized human female. The present report concerns similar estrogen studies on (a) a normal human male and (b) a bilaterally orchiectomized human male. These studies were made to establish a basis for work of a similar nature on human subjects with cancer of the prostate gland. In general, the human male metabolizes therapeutic doses of the natural estrogens in a manner similar to the bilaterally ovariectomized hysterectomized human female. Estriol apparently is not converted to estrone or estradiol, estrone and *a*-estradiol are partially interconvertible and the injection of either leads to the excretion of estriol also, and only a small fraction of the injection material of any of the three estrogens is recovered in estrogenically active forms. The nature and amount of the estrogens excreted are profoundly influenced by (a) the vehicle in which the hormone is administered, (b) the route of administration, and (c) the nature of chemical conjugation of the administered hormone. The presence of the male gonads appears to influence to some degree the manner in which the therapeutic doses of the female hormones are metabolized. From these studies certain conclusions can be drawn regarding the type and amount of estrogenic substances which might prove satisfactory for further studies.

#### 16. A SIMPLE QUANTITATIVE COLORIMETRIC TEST FOR ESTROGENS.

Herman Cohen and Robert W. Bates, E. R. Squibb and Sons, New Brunswick, New Jersey.

The Kober test for estrogens and various modifications thereof, consist of two steps: 1) Initial heating with a small volume of  $H_2SO_4$  mixed with a phenolic compound and 2) dilution with water or aqueous  $H_2SO_4$  and reheating.

Absorption spectrum studies have shown that the presence of phenol, B-naphthol sulfonic acid or thiocol does not change the absorption curves from those obtained using  $H_2SO_4$ , alone, nor are the extinction coefficients appreciably different. The action of phenol is to quench the green fluorescence which affects the visual appearance of the red color but not the response of the photoelectric cell.

Based on these observations the following simple procedure, omitting the phenol reagent, has been developed.

0.4 cc. of an ethanol solution of estrogen is heated with 2.0 cc. of concentrated  $H_2SO_4$  for three minutes in a boiling water bath. Remove tube from bath and add at once 8 cc. of 25%  $H_2SO_4$  (by volume). Stir and heat the solution again for three minutes at 100°C. Cool in tap water and read the color density in a colorimeter using a 510 mu filter. The

color developed is stable at room temperature for four hours. Beer's law holds between the limits of 5 to 60 gamma of estrone.

The intensities of the color produced with estrone, estradiol and estriol differ, so that quantitative determination of each compound in a mixture requires fractionation.

#### 17. THE APPLICATION OF THE ZIMMERMANN AND THE KOBER REACTIONS CONCOMITANTLY TO HUMAN URINE

William T. Salter, Department of Pharmacology and Toxicology, Yale University School of Medicine, New Haven, Connecticut.

In order to detect a possible reciprocal interplay between androgenic and estrogenic effects in the organism it is desirable to measure concomitantly various types of steroid hormone. In the absence of suitable methods for their detection in blood, the estimation of urinary steroids offers a possible approach. Because it is impractical to measure the many derivatives known to exist simultaneously in human urines, it becomes necessary to use procedures which record the net effect of large groups of compounds. In addition, microchemical analysis conceivably may be more significant than biological assay because relatively inactive components like etiocholanolone and estriol may represent forms of hormones which originally played important metabolic roles.

As a first approximation the Zimmermann and Kober reactions have been applied together in metabolic studies varying in duration from 6 to 24 hours. Technically, the definitive color in each case is obscured by the presence of extraneous brown pigments. Instead of attempting complete purification of the chromogens, it is convenient to develop the color after partial purification of the extracts. Thereupon, the undesirable pigments may be removed in large measure by differential solubility in appropriate extracts of varied polarity. In the case of 17-ketosteroids it has not been necessary to use an additional optical correction; but with males and non-pregnant females Kober's pigment must be estimated by optical analysis even after preliminary extraction of the final colored compound. Although the Kober pigment fluoresces strongly when in pure solution, a disturbing quenching may be observed in certain contaminated solutions. Therefore thus far the photoelectric colorimeter has been employed for routine work.

It is convenient to locate the concomitant values derived by such procedures on a rectangular graph depicting 17-ketosteroids as abscissae and estrogenic-equivalents as ordinates. When this procedure is followed certain clinical syndromes occupy characteristic positions on the plot.

(This work was aided by grants from the Navy Task Order VI, N6ori-44, The Donner Foundation and the Jane Coffin Childs Memorial Fund.)

#### 18. THE METABOLIC PATHWAY OF ESTRIOL PRODUCTION IN THE ORGANISM.

Max N. Huffman and Arthur Grollman, from the Southwestern Medical College, Dallas, Texas.

The existence of a reversible equilibrium between estrone and  $\alpha$ -estradiol and the conversion of the former to the latter in the human is well established. The pathway of the conversion of estrone to estriol is still, however, a matter of speculation. It is generally assumed that estrone is enolized and then hydrated to give estriol or that this hypothetical enol compound is formed directly by dehydrogenation of  $\alpha$ -estradiol. A knowledge of the exact course of estrogen metabolism is important for it may lead to the production of more active naturally occurring estrogens as well as to better concepts of the possible role of these compounds in carcinogenesis and in the body economy in general.

An alternative hypothesis to account for the transformation of estrone to estriol is suggested; viz., that estrone is first converted to 6-keto-estrone which in turn is reduced first to 16-keto- $\alpha$ -estradiol and finally to estriol. One of us (M.N.H.) has prepared an epimer of estriol in which both C<sub>16</sub> and C<sub>17</sub> have  $\alpha$ -configurations. This compound has been designated as iso-estriol-A. Reduction of 16-keto-estrone yields four possible stereoisomers, of which three have been prepared. We have determined the estrogenic activities of these compounds as well as of 16-keto-estrone prepared by partial synthesis from estrone. The results permit certain deductions regarding the probable pathway of estriol production in the organism.

#### 19. COLOR REACTIONS OF THE STEROIDS.

Herbert Jaffe, Babette Solomon, and Robert H. Williams. Thorndike Memorial Laboratory, Second and Fourth Medical Services (Harvard) Boston City Hospital, and the Department of Medicine, Harvard Medical School, Boston, Massachusetts.

A systematic survey of various color reactions of the steroids is being made with the principal objective of establishing and evaluating the structural characteristics essential for chromogenicity. Preliminary observations on thirty-seven steroids, comprising bile acids, sterols, androgens, estrogens and corticosteroids, and grouped according to progressive modifications in the nuclear ring substituents and structures, reveal that the following reactions can be associated with specific elements of the steroid structure:

*Meta-Dinitrobenzene and Antimony-Trichloride Reactions.* The *m*-dinitrobenzene reagent is specific for ketonic groups present either in the ring or in the side-chain. On the other hand, the presence of hydroxyl groups is essential for chromogenicity with the antimony-trichloride reagent, but the position of the alcoholic groups modifies the hue, intensity and rate of color development. Thus, for example, the hydroxycholeanic acid yields a positive antimony reaction and a negative *m*-dinitrobenzene reaction, but the ketocholeanic acids react conversely. Ketols vary in their reactivity towards each reagent, depending on the preponderant characteristics and the positions of the substituent hydroxyl and ketonic groups.

It is to be noted further that other polyvalent metals, viz. iron and aluminum (some-what soluble in organic solvents as their chlorides) may replace antimony, effecting similar color changes.

#### 20. CHEMICAL ASSAY OF URINE FOR ADRENOCORTICAL HORMONES IN ENDOCRINE AND NON-ENDOCRINE DISEASES.

William H. Daughaday, Herbert Jaffe and Robert H. Williams.

In determining the quantity of adrenocortical hormones in urine, we have adopted the principle proposed by Lowenstein of measuring the formaldehyde liberated by the oxidation of urinary extracts with periodic acid. However, with Lowenstein's procedure certain substances in the urinary extracts interfere with the color reaction with chromotropic acid. To circumvent this difficulty, it is possible to distill the formaldehyde into a sulfite solution. A pure purple color is then obtained by the reaction with chromotropic acid and this is measured in an Evelyn photocolormeter. We have used this method in 75 determinations. The results have been expressed as milligrams of adrenocortical hormone per 24 hours. Excretion by healthy adults has averaged 1.2 mg./24 hours (range 1-1.5 mg./24 hours). Four patients with Addison's disease averaged 0.5 mg./25 hours. Two cases of Cushing's syndrome were 2.8 and 23 mg./24 hours. In 9 patients with exogenous obesity the excretion was normal, but in two determinations it was above normal. Low values were found in either hyper- or hypothyroidism. Hypopituitarism was as-

sociated with levels intermediate between Addison's disease and normal. Decreased excretion was also found in one patient with Waterhouse-Friderichsen syndrome and in one patient with scurvy.

21. DISAPPEARANCE OF DIABETES MELLITUS ASSOCIATED WITH ACROMEGALY FOLLOWING ACUTE MASTOIDITIS AND BASILAR MENINGITIS.

T. P. Almy and Ephraim Shorr. Russell Sage Institute of Pathology, the Department of Medicine, Cornell University Medical College, and The New York Hospital, New York City.

Complete disappearance of diabetes mellitus was observed in a 40-year old male with acromegaly of 14 years duration, the last 5 being complicated by diabetes and impotence. A 4+ glycosuria was regularly present despite 60 units protamine insulin daily. An oral glucose tolerance test gave the following blood sugar values: fasting, 250; 1 hour, 455; 2 hours, 555 and, 3 hours, 500 mg. %.

He then developed acute right mastoiditis and petrositis, basilar meningitis, retinal hemorrhages, visual field defects and ophthalmoplegia. On the 18th day of the illness, for which mastoidectomy was performed, the urine became sugar-free and fasting blood sugar and sugar tolerance curves had returned to normal levels. He has now remained free of diabetes for 5 years.

The BMR has fallen to -30% with no alteration, however, in serum cholesterol. There is no evidence of adrenal insufficiency. Impotence, associated with urinary 17-ketosteroid values of 2.5 mg., has persisted, except during androgenic therapy. Three years after this episode, the sella was found reduced in size.

The sudden disappearance of diabetes is attributed to partial degeneration of the anterior pituitary, during the acute infectious episode, with a consequent reduction in the elaboration of the diabetogenic principle.

22. TRUE HERMAPHRODITISM: REPORT OF A CASE WITH AN OVOTESTIS, AND ENDOCRINE STUDIES.

John C. Weed (by invitation), A. Segaloff, Wm. Wiener (by invitation) and J. W. Douglas (by invitation). From the Divisions of Gynecology and Medicine, The Charity Hospital of Louisiana at New Orleans and the Alton Ochsner Medical Foundation, New Orleans, Louisiana.

A colored "female," age 36, exhibiting feminine bodily contours and feminine social adjustments, was found to have an enlarged clitoris, masculine facial hair distribution, and abnormal external genitalia. In a large hernial sac, an ovotestis was found associated with an atrophic uterus, left tube and ovary. Biopsy sections of the latter revealed normal ovarian stroma and a small Brenner tumor. Sections of the ovotestis which was completely removed, revealed normal ovarian stroma, definite testicular tubules without spermatogenesis, and large islands of interstitial cells. A rudimentary epididymis and fallopian tube were found in the hernial sac. Preoperative determinations of the gonadotropic hormone and of 17-ketosteroids were made on 24 hour urine collections, and these were repeated postoperatively. The gonadotropic hormones remained essentially unchanged in both series of assays. The 17-ketosteroid determinations were 7.5 and 7.6 mg. per 24 hours preoperatively and from 4.8 to 5.6 mg. per 24 hours postoperatively.

23. TESTOSTERONE IN A CASE OF POLYOSTOTIC FIBROUS DYSPLASIA.

Rita S. Finkler and George M. Colin. From the Endocrine Clinic and Service, Newark Beth Israel Hospital, Newark, New Jersey.

A case of polyostotic fibrous dysplasia observed over a period of several years is reported. The patient was referred for treatment to the Endocrine Service at the age of 13



after 4 years observation in other clinics. The most disturbing symptoms were progressive loss of vision in the right eye due to pressure of fibrous hyperplasia of the maxillary area upon the contents of the right orbit and progressive facial asymmetry. Since the disease is said to be self limiting at maturity, it was decided to attempt hastening maturity by the administration of testosterone propionate, because of the progressively failing vision. After one year of therapy, beginning June 1941, periodic examinations revealed gradual improvement in vision due to regression of bone pathology of the right orbit and a regression of the fibrous dysplasia in other bones of the body. At the last examination, in September 1946, there was almost complete recovery of vision in the right eye and regression of bone pathology in other parts of the body. It is felt that the use of testosterone propionate hastened maturity and the period of recession in this case since prior to treatment it was estimated that regression would not commence until after the age of 15.

24. BILATERAL ARRHENOBLASTOMA WITHOUT MASCULINIZATION, ADENOMA TESTICULARE OF PICK.

Minnie B. Goldberg and Alice F. Maxwell, San Francisco.

*Case Report*

This report deals with a 19 year old girl presenting a paradoxical picture of primary amenorrhea, tall eunuchoid build, *large breasts*, poorly developed external genitalia with rudimentary vagina, congenital absence of uterus and cervix, absent axillary and scant pubic hair and a high urinary gonadotropin titer. The adnexa were not palpable.

Prior to performing a vaginal plastic operation surgical exploration of the pelvis was carried out with the surprising finding of bilateral, poorly developed gonadal structures composed of tissue grossly resembling adrenal cortex. This, on microscopic section, proved to be a highly differentiated type of arrhenoblastoma. Careful search revealed no evidence of ovarian tissue. Both tumors were removed. Bilateral rudimentary structures suggesting the unfused anlage of uterus were also demonstrated.

The source of the estrogens responsible for the breast development remains a mystery. This case brings up many interesting questions concerning sex determination and differentiation involving problems in genetics, endocrinology and intersexuality.

Illustrated with photographs, photomicrographs and slides.

*Papers read by title*

25. SOCIAL AND PSYCHOLOGICAL READJUSTMENT OF A PSEUDOHERMAPHRODITE UNDER ENDOCRINE THERAPY.

Rita S. Finkler, Newark, New Jersey.

An intersex individual, aged 21, raised as a female, failed to develop either male or female secondary sex characteristics at puberty. The patient was asthenic and lacked endurance and energy, but excelled scholastically. The psychological status was of masculine pattern. He was fairly content until the age of 19, when he developed an emotional attachment for a girl and at the same time became aware of an enlarged clitoris. This discovery was somewhat of a shock and produced emotional conflicts resulting in symptoms of anxiety neurosis which lasted for over 2 years until a thorough investigation revealed the individual to be an "unfinished male." The investigations consisted of bio-assays, psychological analyses and exploratory laparotomy. Intensive substitution therapy with various testosterone compounds developed male secondary sex characteristics, and brought about a marked improvement in the patient's general health, energy, endurance and outlook on life. The patient was stimulated to still further scholastic and

intellectual achievement. As long as therapy was continued, the patient's physical and psychological status continued to improve. Interruption in therapy for any appreciable length of time resulted in diminution of energy, endurance and mental alertness. Lantern slides.

26. SOME OBSERVATIONS ON THE UTILIZATION OF A LIQUID CHROMATOGRAM TECHNIQUE IN THE COLORIMETRIC ESTIMATION OF URINARY PREGNANEDIOL.

Benjamin F. Stimmel. From the Rees-Stealy Medical Research Fund, Ltd., San Diego, California.

In our preparation of urinary phenolic residues for chromatographic fractionation of the estrogens, a neutral fraction of the ether soluble material is obtained. This fraction may be utilized for colorimetric ( $H_2SO_4$ ) pregnanediol determinations in a procedure in which the chromatogram technique has been incorporated. Aqueous solutions of pure pregnanediol glucuronidate similarly treated have been found to yield two separate chromatographic fractions which respond to the color reagent. One of these is so weakly bound to the column of activated alumina that it appears in the initial benzene filtrate whereas the second (pregnanediol) requires much stronger eluting agents (2% methanol-benzene). It is hypothesized that a portion of the pregnanediol loses one or more of its hydroxyl groups during hydrolysis of the glucuronidate as has been shown to occur with alcoholic 17-ketosteroids under similar conditions. This partial destruction of the pregnanediol molecule is maximal during hydrolysis by autoclaving and minimal during simultaneous extraction and hydrolysis as described by Astwood et al. The use of the latter hydrolysis technique on our aqueous extracts of the butanol residues permits satisfactory colorimetric estimation of the pregnanediol content of both pregnancy and nonpregnancy urine. It appears that the method is sufficiently sensitive to detect pregnanediol excretion in a bilaterally ovariectomized human female following the injection of 10 mg. of progesterone. Some studies on pregnanediol and estrogen excretion by patients under simultaneous treatment with therapeutic doses of natural estrogens and progesterone will be presented.

27. THE ORAL USE OF CRUDE ADRENAL CORTEX IN THE STIMULATION OF GROWTH OF THE FACE, PARTICULARLY THE CONDYLE OF THE MANDIBLE.

Francis M. Pottenger, Jr., Monrovia, California.

This paper consists of a compilation of the results of a study of a group of patients who have been on adrenal cortical material for periods of two years or more. It encompasses some of our work over a fifteen year period.

Briefly stated, in patients taking the crude adrenal cortex regularly, we have noticed a definite increase in the development of the middle third of the face, but particularly in the length and thickness of the ramus of the mandible, and secondarily in the forward movement of the body of the mandible.

SATURDAY, JUNE 7

9:00 A.M. Viking Room

28. CHEMICAL AND CYTOCHEMICAL STUDIES OF THE RAT'S ADRENAL CORTEX FOLLOWING THE ADMINISTRATION OF PITUITARY ADRENOCORTICOTROPIC HORMONE (ACTH)

Helen Wendler Deane (by invitation) and Grace E. Bergner (by invitation). (Introduced by E. W. Dempsey.) Department of Anatomy and Medicine, Harvard Medical School, and the Medical Clinic, Peter Bent Brigham Hospital, Boston, Massachusetts.

Purified pituitary adrenocorticotrophic hormone (Armour), containing minimal oxy-

toxic activity, was injected into fasting adult male rats at varying doses and for varying periods of time up to twenty-four hours. Hypertrophy of the adrenal glands and atrophy of the thymus were observed with 2½ mg. of ACTH given every six hours for four doses. Adrenal cholesterol was decreased, and liver glycogen was increased.

Adrenals were fixed in formalin, and frozen sections were stained with sudan IV and Schiff's reagent, and unstained sections were studied for fluorescence and birefringence. Positive reactions by these methods characterize ketosteroids (Cf. Dempsey and Wislocki, *Physiol. Rev.* 26: 1, 1946). In ACTH treated animals, birefringence and sudanophilia declined noticeably in the zona fasciculata of the adrenal cortex; whereas fluorescence and the Schiff reaction remained normally intense. Shorter treatment with 10 mg. ACTH or smaller doses of ACTH resulted in similar but less marked changes. These observations indicate that the zona fasciculata of the adrenal cortex is under pituitary control and secretes steroid hormones which influence thymus size and glycogen deposition in the liver.

#### 29. CHANGES IN CIRCULATING LEUKOCYTES INDUCED BY PITUITARY ADRENOCORTICOTROPIC HORMONE (ACTH) IN MAN.

A. Gorman Hills (by invitation), Peter H. Forsham (by invitation), and Clement A. Finch (by invitation.) Department of Medicine, Harvard Medical School, and the Medical Clinic, Peter Bent Brigham Hospital, Boston.

The effect of purified adrenocorticotropin (Armour) upon blood elements was studied in normal subjects and in patients with Addison's disease. Intramuscular injections of 25 mg. of ACTH at 8:00 a.m. was preceded and followed four hours later by blood counts including direct eosinophil enumeration. Absolute polymorphonuclear, lymphocyte, and eosinophil counts four hours after ACTH were expressed as per cent of initial values.

In eight normal subjects ACTH administration induced an increase in neutrophils (90 per cent), a decrease in lymphocytes (40 per cent), and a decrease in eosinophils (78 per cent); whereas similar treatment in eight patients with Addison's disease was accompanied by only a slight increase in neutrophils (15 per cent) without a significant change in lymphocytes or eosinophils. Administration of 20 mg. of Compound F (17-hydroxycorticosterone) intramuscularly in four of these patients was followed by increased neutrophils (129 per cent), decreased lymphocytes (53 per cent), and decreased eosinophils (76 per cent). With Compound A (11-dehydrocorticosterone), 50 mg., these changes were minimal. No effect was observed with 11-desoxycorticosterone (10 to 20 mg.).

It follows that an increase in adrenal cortical hormone level is accompanied by significant hematological changes, viz. an increase in circulating neutrophils and a decrease in lymphocytes and eosinophils.

#### 30. RESULTS OF ADMINISTRATION OF ANTERIOR PITUITARY ADRENOCORTICOTROPIC HORMONE TO A HUMAN SUBJECT.

Harold L. Mason, Marschelle H. Power, Edward H. Ryncarson, Letizia C. Ciaramelli, Choh Hao Li and Herbert M. Evans. Mayo Foundation, Rochester, Minnesota, and Institute of Experimental Biology, University of California, Berkeley, California.

A young woman was maintained on a constant diet while receiving intramuscularly 25 mg. of adrenocorticotropic hormone daily for six days, 50 mg. for six days and 100 mg. for 11.5 days, given in five divided doses each day. Excretion of sodium, potassium, chloride, phosphorus, total nitrogen, creatine, creatinine, 17-ketosteroids and cortin-like

substances was measured daily and that of pregnanediol, estrogen and gonadotropic hormone at intervals. Levels of sodium, potassium, chloride,  $\text{CO}_2$ -combining power, cholesterol, ascorbic acid, protein, hemoglobin and alkaline phosphatase, the hematocrit and erythrocyte and lymphocyte counts were determined at intervals. During the first two periods the only significant change was an increase in the excretion of cortin-like substances from an average control value of 0.180 mg. to average values of 0.255 and 0.387 mg., respectively. When 100 mg. per day was given the excretion of cortin-like substances increased to 1.44 mg. on the tenth day and the excretion of 17-ketosteroids increased from an average control value of 4.85 mg. to 15.5 mg. on the tenth and eleventh days. There was also a slightly increased nitrogen excretion, a decreased creatine excretion and a pronounced fall in hemoglobin. Other changes were minimal and of equivocal significance.

### 31. METABOLIC CHANGES FOLLOWING THE ADMINISTRATION OF PITUITARY ADRENOCORTICOTROPIC HORMONE (ACTH) IN MAN.

George W. Thorn, F. T. Garnet Prunty (by invitation), and Peter H. Forsham (by invitation). Department of Medicine, Harvard Medical School, and the Medical Clinic, Peter Bent Brigham Hospital, Boston.

Purified adrenocorticotropin (Armour), 2 gamma giving a positive test by the method of Sayers and Sayers, with oxytocic activity of 0.12 units per mg., was administered to a male aged forty-six with partial reduction in pituitary function. On a constant dietary regimen, the patient was given 10 mg. of ACTH in saline (pH 9) every six hours intramuscularly for six days (40 mg. daily). Peripheral vasoconstriction, bradycardia, and abdominal cramps occurred following each injection.

The following changes were observed:

Increased 11-oxy-steroid excretion (sevenfold)

Increased 17-ketosteroid excretion (fivefold)

Increased uric acid excretion (102 per cent)

Increased total nitrogen excretion (45 per cent)

Transitory increase in potassium and phosphorus excretion

Decreased sodium excretion (68 per cent)

No change in creatinine excretion

Increased fasting blood sugar level

Elevated glucose tolerance curve

Decreased serum inorganic phosphorus

Increased serum sodium and carbon dioxide combining power

Decreased plasma protein concentration

Decreased serum uric acid level

Increased polymorphonuclear leukocytes

Decreased lymphocytes and eosinophils

Control studies with pituitrin failed to show any of the above changes with the exception of hemadilution.

### 32. URINARY URIC ACID-CREATININE RATIO FOLLOWING ADMINISTRATION OF PITUITARY ADRENOCORTICOTROPIC HORMONE (ACTH) AS A SIMPLE TEST FOR ADRENAL CORTICAL FUNCTION.

Peter H. Forsham (by invitation), F. T. Garnet Prunty (by invitation), and George W. Thorn. Department of Medicine, Harvard Medical School, and the Medical Clinic, Peter Bent Brigham Hospital, Boston.

Earlier studies indicated that an increase in uric acid excretion with a rise in uric

acid-creatinine ratio constituted one of the most consistent findings which followed the administration of the 11-oxy adrenal steroids (Compounds A and F) in man. The same changes have been found to accompany the administration of adrenocorticotropin (Armour) in ten normal subjects, in five patients with miscellaneous diseases without evidence of adrenal insufficiency, and in two patients with anterior pituitary deficiency.

In contrast, it was noted that the maximum increase in uric acid-creatinine ratio following ACTH therapy in eight patients with Addison's disease did not exceed the minimum increase observed in the control group; whereas the administration of either Compound F (20 mg.) or Compound A (50 mg.) induced a significant increase in the uric acid-creatinine ratio in these patients. The presence of renal impairment limits this test for adrenal cortical *reserve* function.

### 33. FURTHER STUDIES ON THE PROTECTIVE POWER OF ADRENAL PREPARATIONS AGAINST BACTERIAL TOXINS.

Lena A. Lewis and Irvine H. Page. From the Research Division of the Cleveland Clinic Foundation, Cleveland, Ohio.

Determination of the relative protective power of adrenal extracts and steroids against typhoid vaccine in adrenalectomized rats has been shown to be a satisfactory method of estimating toxic protection power of hormones.

It seemed desirable to determine whether these substances would be effective in protecting adrenalectomized rats against other types of toxin and to compare the minimal lethal dose (M.L.D.) of the toxin as determined on normal and adrenalectomized rats as a possible means of evaluating the relative importance of adrenal activity in different infectious processes.

Streptococcus toxin, diphtheria toxin, crystalline tetanus toxin, and typhoid vaccine were tested. The streptococcus toxin was non-lethal to either normal or adrenalectomized rats in doses as large as 2.5 ml. of concentrated toxin. The M.L.D. of diphtheria toxin was 5 times as great for normal as adrenalectomized rats, while that of typhoid vaccine was 25 times as great for the normal. No difference could be demonstrated between the M.L.D. of crystalline tetanus toxin for normal and adrenalectomized rats, 60 units killing 100 per cent in both groups.

In adrenalectomized rats, larger doses of adrenal extract or 11-dehydrocorticosterone acetate were required to protect against 1.33 M.L.D. of diphtheria toxin than against 1.33 M.L.D. typhoid. No protective power of adrenal extract against tetanus toxin could be demonstrated. Studies to determine possible protective power of adrenal extract against bacterial peritonitis in mice are being carried out.

### 34. EFFECT OF TESTOSTERONE UPON THE EXCRETION OF GLYCOGENIC CORTICOIDS.

E. H. Venning and J. S. L. Browne. From the McGill University Clinic of the Royal Victoria Hospital, Montreal.

The excretion of urinary glyco-genic corticoids was followed in a series of 8 individuals before and after the administration of testosterone propionate or of methyl testosterone. Of these cases two were normal individuals, two were patients with Cushing's Syndrome, and the others suffered from hirsutism, diabetes, and one was a patient with lupus erythematosus disseminatus.

The administration of testosterone caused a lowering in the excretion of glyco-genic corticoids in all cases with the exception of one of the patients with Cushing's Syndrome. In no case were the glyco-genic corticoids depressed below the limit of normal values.

### 35. HEPATO-RENAL FACTORS IN CIRCULATORY HOMEOSTASIS: XVIII RELATION OF ADRENALS TO FORMATION OF A RENAL VASO-EXCITOR PRINCIPLE.

B. W. Zweifach, Ephraim Shorr, Silvio Baez and S. Rosenfeld. From the Depart-

ment of Medicine, Cornell University Medical College and The New York Hospital, New York City.

An inter-relationship has been demonstrated between the adrenals and the renal mechanism which elaborates the vaso-excitor principle (VEM) which we have detected in blood during the compensatory phase of shock and the acute phase of renal hypertension.

VEM is produced by kidneys from normal animals under reduced oxygen tensions *in vivo* and *in vitro*, and by kidneys of hypertensive dogs, both anaerobically and aerobically. It is detected by its potentiating effect on the response of the terminal mesentery blood vessels to epinephrine.

Following bilateral adrenalectomy (rats, rabbits, dogs) the renal capacity to form VEM became progressively impaired. Three procedures were employed: (1) adrenalectomy; (2) adrenalectomy plus high NaCl intake; (3) adrenalectomy plus NaCl and DCA (0.1 mg./kg. daily).

The mesenteric blood vessels of adrenalectomized rats exhibited a progressive unresponsiveness to epinephrine as adrenal insufficiency developed. *In vitro* incubation of kidney slices from adrenalectomized animals revealed progressive impairment of VEM formation even in animals maintained on high salt intakes. Kidneys removed from adrenalectomized animals maintained on NaCl and DCA for 10-15 days postoperatively behaved like normal kidneys in respect to VEM formation.

### 36. THE ROLE OF THE ADRENAL CORTEX IN PROTEIN CATABOLISM FOLLOWING TRAUMA.

C. G. Toby and R. L. Noble (introduced by J. B. Collip). Research Institute of Endocrinology, McGill University, Montreal, Canada.

Trauma (limb clamping) in the rat caused a marked excretion of urinary nitrogen. This was not increased by repeated cortin administration.

Nitrogen excretion in the normal rat was only moderately increased after treatment with a total dose of cortical extract equal to 240 dog units administered over 8 hours.

Nitrogen excretion in the adrenalectomized rat varied depending on the time after operation. It was slightly elevated by 4 days, and had returned to normal after two weeks.

Trauma when applied to the adrenalectomized rat did not cause any increased loss of urinary nitrogen.

Suitable treatment of traumatized rats with cortical extract allowed a practically normal response in nitrogen excretion to occur. The same treatment did not cause increased nitrogen loss in untraumatized adrenalectomized rats. Desoxycorticosterone acetate or hypertonic saline therapy were ineffective.

The experimental results indicate that following trauma, secretion of cortin is not responsible for the increased loss of nitrogen. On the other hand, nitrogen loss does not occur in the absence of the adrenal glands unless replacement therapy with cortin is administered. Adrenal cortical secretion is apparently necessary for the mechanism concerned to operate normally although such secretion per se is not responsible for the increased protein catabolism.

### 37. A NEW HORMONE OF THE ADRENAL CORTEX.

Frank A. Hartman, Katharine A. Brownell and Jonathan S. Thatcher. Department of Physiology, The Ohio State University, Columbus.

The movement of fat from the reserves of the liver during inanition depends upon the adrenal cortex since it fails to occur if the latter is destroyed. The substance responsible, which we propose to call the "fat factor," has been separated from the sodium and carbohydrate factors by chromatographic adsorption. It causes deposition of fat in the

liver of an adrenalectomized animal during starvation, while the other factors do not. Likewise, the crystalline compounds, corticosterone, 11-dehydrocorticosterone and 11-dehydro-17-hydroxycorticosterone, are unable to do this. Further evidence that the carbohydrate and fat factors are distinct has been obtained from animals whose adrenals have been enucleated. The output of these factors reaches a point above normal one or more weeks after enucleation. However, the maximum increase for the fat and carbohydrate factors develops at widely different times.

### 38. STUDIES ON OBESITY.

Robert H. Williams, William H. Daughaday, Walter F. Rogers, Jr., Samuel P. Asper, Jr., and Beverly Towery. Thorndike Memorial Laboratory, Second and Fourth Medical Services (Harvard) Boston City Hospital, and the Department of Medicine, Harvard Medical School, Boston, Massachusetts.

Since obesity is often of major consequence and is not, on the whole, satisfactorily controlled, we have used recently available methods in the reinvestigation of certain phases of this aged problem.

The protein-bound iodine of the plasma, determined in 24 patients with marked obesity but with basal metabolic rates above  $-7$  per cent, was found to be normal, in 33 per cent, but in 67 per cent it might be considered subnormal, varying from 1.3 to 4.4 gamma per 100 cc. of plasma, with an average of 3.3 gamma. None of the patients had clear-cut clinical evidence of hypothyroidism and none had a goiter.

The excretion of ketosteroids in the urine was subnormal in 9 of 13 patients tested. The "corticosterone" excretion was found to be essentially normal. The glucose-insulin tolerance test yielded evidence of resistance to insulin in each of 8 patients.

Several weeks after removing each of the endocrine glands of rats, estimations of the "neutral fat" of the carcass showed no appreciable increase in the fat content. Moreover, no striking fat deposition resulted from the administration of large doses of the hormones in common use.

Over 100 markedly obese individuals were treated with from 1 to 7 compounds, including 2-amino-6-methylheptane, dextroamphetamine, 1-cyclohexyl-2-aminopropane, p-hydroxy-alpha-methylphenethylamine hydrobromide, laevo-amphetamine, and d-desoxyephedrine hydrochloride. Of these compounds, the first two were the most satisfactory. The anti-obesity effect was apparently due to the decreased hunger and the increased exercise which the drugs produced.

### *Papers read by title*

### 39. PELLET THERAPY WITH DESOXYCORTICOSTERONE ACETATE IN ADRENAL CORTICAL INSUFFICIENCY.

George F. Koepf and (by invitation) Raymond Kibler. Departments of Medicine and Physiology, University of Buffalo, School of Medicine.

During the five year period (1941-1946) thirty-two patients with adrenal cortical insufficiency were treated with subcutaneously implanted pellets of desoxycorticosterone acetate (Ciba). Although sodium chloride in addition to the normal dietary intake was given during an assay period in which the daily dose of D.O.C.A. in oil was being ascertained, supplementary sodium chloride was seldom found necessary after implantation. Several patients were given adrenal cortical extracts during episodes of acute infections, but in only two cases was it necessary to supplement the therapy for prolonged periods because of severe, repeated attacks of hypoglycemia.

Data to aid in determining the number of pellets was obtained and may be summarized as follows:

- (1) The average daily hormone absorption from a 125 mg. pellet (Ciba) by a human is 0.35 mg.
- (2) Approximately 60% of the daily dose of D.O.C.A. in oil is required if the D.O.C.A. is given in pellet form.
- (3) To determine the number of pellets of D.O.C.A. to implant the following formula is useful:

$$\text{pellets needed} = \frac{\text{daily dose D.O.C.A. in oil} \times 0.60}{0.35}$$

- (4) The average duration of each implant was 334 days.

Eighteen of the thirty-two patients were diagnosed as non-specific adrenal cortical insufficiency, and two (11%) of these had died. All of the nine patients with tuberculosis of the adrenal cortex have died, however, some prolongation of life in most of these cases was noted. One (20%) of the five patients with adrenal cortical insufficiency secondary to panhypopituitarism has died.

#### 40. RELATIONSHIP OF SEX STEROIDS TO THE ADRENAL GLANDS OF HAMSTERS AND RATS.

Herbert S. Kupperman and Robert B. Greenblatt.

Sex dimorphism in the size of the adrenal has been reported in the rodents with the female animal exhibiting the heavier adrenal. However, data in the golden hamster revealed a reversal of this sex dimorphism whereby the adrenal of the male hamster was larger than that of the female. Autopsy performed on animals 15 to 600 days of age showed that the adrenal weights were comparable in both sexes until the 30th day of life at which time a sex difference became evident. The adrenal weight in both the male and female animals plateaued at 100 days of age with the weight of the male adrenals recorded at 65% greater than those of the female. The increase in size of the male adrenal coincided with the beginning of androgenic activity as evidenced by the appearance of dimorphic pigmentation in the male and the period of rapid growth of the prostate and seminal vesicles. Administration of sex hormones to castrate and normal male and female hamsters failed to demonstrate a specific action of the sex steroids. The effects of estrogen and progesterone upon the adrenal glands of albino rats were noted after estrogen administration. With the doses employed no hypertrophy of the adrenals was noted after estrogen administration. There was a variation in response to progesterone. The results, together with the morphological findings, are described and discussed in the light of the adrenal-gonad-pituitary relationship.

#### 41. CORRELATIONS OF BIOCHEMICAL AND HISTOLOGICAL CHANGES IN THE ADRENAL CORTX IN VARIOUS TYPES OF DISEASE.

Walter F. Rogers, Jr., and Robert H. Williams. Thorndike Memorial Laboratory, Second and Fourth Medical Services (Harvard), Boston City Hospital, and the Department of Medicine, Harvard Medical School, Boston, Massachusetts.

Certain biochemical and physiological changes in the adrenal glands of 103 (selected) patients dying of a wide variety of diseases were compared with the glands of normal individuals who experienced instantaneous or sudden death. The cholesterol content of the adrenal glands was compared with observations made of the tissue with polarized and ultraviolet light. Comparisons were also made of tissues stained with phenylhydrazine, Sudan IV, and phloxine-methylene blue. It is concluded that a close correlation exists in each of these methods of study and while they give an indication of the total amount and distribution of the lipid deposition in the adrenal cortex, they do not demonstrate the amounts of adrenal hormones present, but they can be used to indicate the functional reactivity of the adrenal cortices in various types of disease.



2:00 P.M. *Viking Room*

42. THE USE OF HYPERTONIC SALINE INFUSIONS IN THE DIFFERENTIAL DIAGNOSIS OF DIABETES INSIPIDUS AND PSYCHOGENIC POLYDIPSIA.

Anne C. Carter and Jacob Robbins (introduced by Ephraim Shorr). From the Department of Medicine, Cornell University Medical College and The New York Hospital, New York City.

Hickey and Hare (*J. Clin. Investigation* 23: 768, 1944) have shown in animals and in a small series of normal human subjects that, after adequate hydration, infusion of 2.5% NaCl (0.25 cc./kg./min. in man) result in reduction in urine flow attributable to an anti-diuretic factor of neurohypophyseal origin. This effect was absent in experimental and in two cases of human diabetes insipidus.

Additional evidence of the value of this procedure for differentiating diabetes insipidus from psychogenic polydipsia is provided by studies on a larger series of human subjects, using urine flow as the index. Nine normal hydrated subjects showed a marked reduction in urine flow during infusion of 2.5% NaCl. This reduction was found not to be due to spontaneous decrease in water diuresis nor to saline per se, and could be prevented by overhydration.

Of ten patients with polyuria, two showed reduction in urine flow during infusion and their subsequent spontaneous decrease in urine volume established the psychogenic basis for the polydipsia. The remainder exhibited sustained diuresis during the infusion and a prompt reduction in urine flow following 0.1 U Pitressin intravenously indicating true diabetes insipidus. In the absence of renal disease, this test provides for differentiation between psychogenic polydipsia and diabetes insipidus.

43. EFFECT OF AQUEOUS TESTICULAR EXTRACTS ON GROWTH AND DEVELOPMENT OF SPONTANEOUS MAMMARY TUMORS IN THE AGING BITCH.

F. X. Gassner. Colorado Agricultural Experimental Station, Fort Collins, Colorado.

An aqueous testicular extract of fresh beef testes, devoid of lipoids and essentially so of protein, was assayed for androgenic properties in intact and castrated male rats, and for estrogenic potency in castrated female rats. Each cc. of the extract represented 10 Gm. of fresh testes.

The sex organs of castrates of both sexes failed to show any response. The seminal vesicles, prostates and testes of the intact males were reduced in weight up to 46 per cent.

Nine aging female dogs with spontaneous mammary tumors received intramuscularly 10 cc. aqueous testicular extract weekly for up to eight weeks. Biopsies were taken at the start and at the end of treatment. Changes in the size of the tumors were photographically recorded weekly. In seven dogs the rapid growth of the tumors was not only arrested but their size was reduced as much as 86 per cent. The initial biopsies showed the presence of adenocarcinomas grades 1 to 3. After 6 weeks treatment extensive hyalinization of the stroma and necrosis and reduction of the tumorous parenchyma were evident. Decided improvement in behavior and well-being of the dogs was noted.

Gynecomastia in one intact male dog and in one castrated male cat subsided after 4 weeks treatment. The probable mechanism involved is discussed.

44. CONJUNCTIVAL AND CORNEAL LESIONS IN HYPERCALCEMIA.

John Eager Howard and Frank B. Walsh. From the Departments of Medicine and Ophthalmology, the Johns Hopkins University and Hospital, Baltimore, Maryland.

In the past few years we have seen, in approximately 50 per cent of our patients with

hypercalcemia, distinctive phenomena in the eyes which are believed to be of diagnostic importance and theoretical interest. Similar lesions have been observed by colleagues at the Massachusetts General Hospital. The crystals cannot be identified with the naked eye so that examination with the slit lamp is necessary. In the deep conjunctiva of the palpebral fissure area are small glass-like particles. There is likely to be some redness, but this may not be pronounced; and there may or may not be complaint of conjunctival irritation. The crystals may be numerous or few. In one instance here and in several instances at the Massachusetts General Hospital, the superficial layers of the cornea have been involved presenting an appearance resembling band-shaped keratitis. Biopsy of one case was studied at the Johns Hopkins University and the crystals reported to be calcium phosphate. The crystals are clear and not at all like the grayish patches sometimes seen extending superficially over a pterygium. The lesions have been seen by us in instances of hyperparathyroidism and vitamin poisoning. In some instances the deposits have disappeared after the metabolic disturbance had been corrected.

Why the conjunctival and corneal membranes should be so involved is not clear. The factor common to our cases seems to be hypercalcemia, the serum phosphorus levels having been normal, high and low. Search for such lesions by ophthalmologists may uncover cases with hypercalcemia which would otherwise have been overlooked by the internist.

45. A SECONDARY SEXUAL CHARACTER THAT DEVELOPS IN AN ORGAN COMMON TO BOTH SEXES BUT NORMALLY ONLY IN MEN. WITH A DISCUSSION OF THE RELATION OF THIS CHARACTER TO ENDOCRINE STIMULATION.

James B. Hamilton, Long Island College of Medicine, Brooklyn, New York.

In organs present in both sexes only relative differences in secondary sex characters have been described. That is, a character is developed to a greater extent or occurs more commonly in one sex. Some interest attaches, therefore, to a masculine trait which distinguishes *absolutely* between normal men and women. This character is the growth of terminal (coarse) hairs on the external ear.

Three factors control development of this trait: aging, endocrine stimulation (apparently androgenic) and genetic predisposition. Large auricular hairs grow in Caucasian men after the 24th year and increase in frequency and coarseness until by the 55th year 75 per cent of men exhibit this character (229 men studied). Terminal hairs were not observed in a series of 105 women and 50 eunuchs. Auricular hairs appeared in three of five eunuchs receiving androgenic treatment and in three of five women with pronounced virilism.

Pronounced hairiness depends more upon age and inheritance than any exceptional degree of androgenic stimulation. No terminal auricular hairs were observed in undoubtedly virile men among 54 American Indians, 84 Chinese and certain Caucasian families. Titters of urinary steroids in 57 normal Caucasian men bore no quantitative relationship to the amount of auricular hair.

46. THE USE OF METHYL TESTOSTERONE AND TESTOSTERONE PROPIONATE IN PRE-MATURE INFANTS.

E. Kost Shelton and Jerome S. Mark. From the University of Southern California and the Shelton Clinic, Los Angeles, California.

Following a preliminary report by E. Kost Shelton, M.D., and Arthur E. Varden, M.D., before the Association for the Study of Internal Secretions, Saturday, June 29,

1946, the experimental use of testosterone, in various forms, on premature infants was continued at the County Hospital in Los Angeles.

Only premature babies below two thousand grams were employed in this study. Every third child was given methyl testosterone by mouth, every third child testosterone propionate by hypodermic, and every third child given nothing by way of specific treatment. The basic care of all these children was the same.

While all of the data are not yet available and the work continues, there seems to be a significant difference in 1) survival, and 2) weight increase in those children treated by testosterone preparations over those not so treated. The results continue to be promising.

New methods of administration of the hormones have been devised.

#### 47. EQUINE PITUITARY GONADOTROPIN AND ANTIHORMONE FORMATION.

James H. Leatham and A. E. Rakoff. From the Bureau of Biological Research, Rutgers University, New Brunswick, N. J., and the Department of Obstetrics and Gynecology and the Endocrine Division, Jefferson Medical College and Hospital, Philadelphia.

Equine pituitary gonadotropin (Squibb) has been studied to evaluate it in terms of antihormone formation in cases subjected to somewhat extended therapy. Thirteen women suffering from anovulatory sterility or functional menstrual disorders and four hypogonadal males have been studied for antihormone formation. All cases exhibited diminished urinary gonadotropins. In general, the females received 50 units twice weekly during two weeks of each month over a 2-5 month period. The males received 50 units twice weekly for 3-5 months. Antihormones against equine pituitary developed in three women all of whom had been treated for three months and in three males in 3-4 months of therapy. No inhibitory substances existed prior to therapy. Antihormones against equine pituitary were also inhibitory against pregnant mare serum gonadotropin. However, two cases in which the serum lacked anti-equine pituitary substances were able to inhibit pregnant mare serum.

Cases exhibiting a prompt response to equine pituitary were not studied for antihormone formation but of those here presented the cases of functional menstrual disorders exhibited a good response, the males a moderate response, and 3 of 7 cases of anovulatory sterility exhibited improved temperature charts.

Equine pituitary gonadotropin appears to have a distinct stimulating action on the human gonad but also elicits antihormone formation.

#### 48. STUDIES IN CASES OF PITUITARY TUMORS.

K. E. Paschkis, A. Cantarow and A. E. Rakoff. Jefferson Medical College and Hospital, Philadelphia.

Studies are reported on a number of cases of chromophobe adenomas and of parapituitary tumors such as craniopharyngiomas.

The picture of hypopituitarism includes the effects of lack of gonadotropin-, thyrotropin- and adrenocorticotropin secretion. The resulting deficiencies were studied by appropriate methods, in a number of cases both before and after operation.

In insulin tolerance tests "hypoglycemia-irresponsiveness" with prolonged severe hypoglycemia was observed with conspicuous absence of clinical manifestations usually present with severe prolonged hypoglycemia.

Impairment of salt metabolism was present in a number of cases. This indicates that, at least in the human, adrenocorticotropic regulation of the adrenal cortex includes salt-water-metabolism contrary to the claim that the latter function is independent of pituitary regulation.

In one case of pituitary tumor the presence of which was indicated by x-ray of the

sella and by visual field examination, there were hypogonadism (absent gonadotropin excretion, amenorrhoea, atrophic endometrium) and lactating breasts. No other functional abnormalities were present. This patient has not as yet been operated upon, and the nature of the tumor is therefore not known.

#### 49. CARBOHYDRATE APPETITE OF NORMAL AND HYPERTHYROID RATS AS DETERMINED BY THE TASTE-THRESHOLD METHOD.

Curt P. Richter. Psychobiological Laboratory, the Johns Hopkins Hospital, Baltimore, Maryland.

Fifty-four normal and 28 hyperthyroid rats were tested with the taste-threshold method for their appetites for 6 common sugars: maltose, glucose, sucrose, fructose, lactose and galactose. Hyperthyroidism did not change the taste thresholds nor the maximum preference concentrations of these 6 sugars. It did produce a great increase in the appetite for the sugars. The hyperthyroid rats took about twice as much of each of the sugars as did the normal animals. Further, they definitely preferred certain concentrations of the sugars to the stock diet.

Both normal and hyperthyroid rats showed the greatest appetite for the corn sugars, maltose and glucose, the smallest appetite for the milk sugars, lactose and galactose, and intermediate appetites for the cane sugars, sucrose and fructose. The results indicated that the appetites varied directly in proportion to the ability of the rats to assimilate the sugars. It was concluded that hyperthyroidism produces a great increase in the ability of the rats to assimilate carbohydrates.

#### 50. THE EFFECT OF HYPOTHYROIDISM ON MENSTRUATION. W. O. Thompson, P. K. Thompson and E. M. Jeppson, University of Illinois College of Medicine, Chicago, Illinois.

A study of spontaneous myxedema developing in adult life shows that it may produce an increase, a decrease, or a complete absence of menstrual flow. Myxedema developing in adult life as a result of subtotal thyroidectomy often produces an increase in flow but does not seem to produce a decrease. The increase in menstrual bleeding with hypothyroidism may at times be very great, simulating an incomplete abortion and producing a secondary anemia.

Regardless of the type of menstrual disturbance, the administration of thyroid in a suitable dose may correct it. However, when there is a complete absence of menstruation, it may fail to return in some instances following the administration of desiccated thyroid even though the patient is still below the age of the menopause.

The hypothesis that excessive bleeding is the result of primary hypothyroidism while oligomenorrhea and amenorrhea are associated with hypothyroidism secondary to hypopituitarism is not supported by the fact that both types of menstrual irregularity in patients with myxedema may be corrected by the administration of thyroid. The failure of amenorrhea to disappear with thyroid feeding may point to its pituitary origin.

#### 51. TWELVE CASES OF METASTATIC THYROID CARCINOMA STUDIED WITH RADIOACTIVE IODINE.

S. M. Seidlin, E. Oshry and A. A. Yalow. Montefiore Hospital, New York, N. Y.

After the administration of tracer doses of radioactive iodine to twelve patients with thyroid carcinoma, the metastases were studied for uptake of the isotope by means of the Geiger-Mueller counter. Six cases were positive (metastases showed uptake of radioiodine), three were negative and three questionable. The radio-autographs were positive in seven, negative in the three and questionable in two. The possible correlation of histological structure of the tumors and radioiodine uptake was attempted. The effect of pre-

treatment with thyrotropic hormone and thiouracil on radioiodine uptake by the metastases was also studied. Radioiodine concentration in the blood and urinary excretions were carried out in all cases. All "positive" cases received or are receiving therapeutic doses of radioactive iodine.

52. GOITER ON AN IODINE-FREE DIET GROWN BY HYDROPONICS, AND EXCLUDING ANY GOITER NOXA.

J. F. McClendon and Wm. C. Foster. Hahnemann Medical College, Philadelphia.

In order to produce an iodine-free diet and exclude a goiter noxa we grew a diet by hydroponics in a disinfected greenhouse with disinfected water and chemicals in a goiter-free region. Air was pumped through a carbon filter. Six litter-mate rats from a colony that had been goiter-free for 6 years were put at weaning on the diet of 40 per cent sunflower seed, 2.8 per cent soy beans, 40 per cent sucrose, 0.8 per cent NaCl and 16.4 per cent corn oil. Three of the rats were given water redistilled from alkali to drink and at the end of 73 days had goiters weighing 39, 42 and 41 mg. per 100 Gm. body weight. The other 3 rats were given water containing 10 parts per million of iodine and had normal thyroids weighing 10 mg. per 100 Gm. body weight. Since the rat cages were enclosed in a cellophane covered frame and no goitrous animals or humans had access to the we believe that so called goiter noxa is excluded. The goiters were 4 times as large as normal thyroids and twice as large as goiters in rats from a goitrous colony fed in a goiter region on a very low iodine diet.

53. MITOTIC ACTIVITY AND WOUND HEALING IN THE CORNEAL EPITHELIUM OF RATS TREATED WITH THIOURACIL.

Walter Fleischmann and Alfred Breckler. Clinical Physiology Section, Medical Division, Edgewood Arsenal, Maryland, and Department of Pediatrics, Johns Hopkins University, Baltimore, Maryland.

The corneal epithelium of the rat has been shown to be a suitable test object for mitotic and wound healing activity. (Buschke, Friedenwald and Fleischmann, *Bull. Johns Hopkins Hosp.* 73: 143, 1943; Friedenwald and Buschke, *J. Cell. and Comp. Physiol.* 23: 95, 1944.) We have compared mitotic counts in flat preparations of corneae of rats treated with thiouracil with those of litter-mate controls. A number of animals were given 5 mg. of colchicine per kg. of body weight 4 hours before killing them, others were killed without previous treatment with colchicine. The number of mitoses as estimated from the colchicine experiments was significantly reduced in the rats treated with thiouracil. In the experiments without colchicine the counts were not reduced in the rats treated with thiouracil. This indicates that not only the onset of mitosis, but the duration of the mitotic cycle is slowed down in thiouracil treated rats. Superficial wounds of the cornea heal in the same time in hypothyroid rats as in normal controls.

*Papers read by title*

54. CLINICAL MANIFESTATIONS IN FORTY CASES OF MYXEDEMA.

David Schwimmer, Mildred Vogel, and Thomas H. McGavack. From the New York Medical College, Metropolitan Hospital Research Unit.

Data are presented on forty cases of myxedema seen during the past seven years in both hospital and office practice. Observation has varied from 12 hour antemortem to over five years.

\*Stress is laid on the presenting clinical manifestations, chiefly as they concern proper diagnosis. It is felt that far too often the diagnosis of myxedema is missed primarily because it is not entertained as a possibility. An important contributing factor in such

failures of recognition of the disease is that the diagnosis generally is based on the classical findings of obesity, dry skin, coarse hair, hoarse voice, apathy, etc.

While it is true that these classical manifestations are unmistakable, such standard findings may be absent. In 20-30 per cent the myxedematous status was masked by symptomatology of cardiac difficulties. "Nervousness," rather than apathy and com-  
placence, was prominent in over 60 per cent.

The cases presented are divided into four groups:

- I. Those with classical symptomatology.
- II. Those with predominantly cardiac manifestations.
  - a. cardiac symptoms without congestive failure.
  - b. cardiac symptoms with congestive failure.
  - c. cases of coronary occlusion.
- III. Those with atypical symptomatology.
- IV. Those without symptoms.

Emphasis is placed on the fact that cardiac failure may raise the basal metabolic rate to normal or above; also, that the serum cholesterol levels may be unexpectedly normal, especially with chronic passive congestion of the liver incident to right heart failure.

#### 55. THIOURACIL IN THE TREATMENT OF HYPERTHYROIDISM COMPLICATING PREGNANCY AND ITS EFFECTS ON THE FETUS.

M. James Whitelaw. From the Department of Obstetrics and Gynecology, Southwestern Medical College.

The relative infrequency of hyperthyroidism as a complication of pregnancy is discussed giving the statistical figures from both European and American sources. A severe hyperthyroid pregnant white female in the 26th week of gestation was treated with thiouracil up to and through the puerperium. She presented the classical clinical as well as laboratory findings characteristic of Graves disease. Her BMR was +65. Under thiouracil therapy .4 gram per day given in divided doses plus general supportive measures, she was symptom free at the time of her delivery. Her BMR had fallen within normal range as had her pulse, blood chemistry and circulation time. An anencephalic monster was delivered which died 6 hours postpartum. Autopsy revealed a thyroid gland that appeared both microscopically and histologically normal. The weight and iodine content of the thyroid were both well within normal limits.

#### 56. THE PERMANENCY OF ALLOXAN DIABETES AND THE STRUCTURE OF THE PANCREATIC ISLETS FOLLOWING CERTAIN EXPERIMENTAL PROCEDURES.

Ralph G. Janes, Department of Anatomy, College of Medicine, State University of Iowa, Iowa City.

When alloxan is given to the rat either subcutaneously, intraperitoneally, or intrasplenically, there is considerable variation in the severity of the resulting diabetes. In mildly diabetic animals (blood sugar up to 250 mg. % and urine volume below 100 cc. daily) the diabetic symptoms usually disappear in 4-6 weeks. In severely diabetic rats (blood sugar levels up to 900 mg. % and urine volumes up to 300 cc. daily) the diabetes remains severe until death. Although it is not always possible to correlate the severity of the diabetes with the amount of islet damage, in general the more severe the diabetes, the more extensive is the islet destruction. When suitable quantities of alloxan are administered the islets lose their brilliant staining qualities, cell boundaries become indistinct, and many of the Beta cells exhibit necrotic changes. Furthermore, islet cell nuclei become more abundant at the periphery, resulting either from shrinkage of the cytoplasm of certain islet cells or perhaps from cell division. In 3-4 weeks following the

injection of alloxan, some of the Beta cells which are not destroyed regain their staining qualities and connective tissue replaces those which are destroyed. In an attempt to modify further the islets following this period, the animals were subjected to several procedures which have been shown to alter carbohydrate metabolism, namely, diets high in niacin, diets with large amounts of thiamine, high fat diets and thiamine deficient diets. No further alteration in the islets has been noted following these procedures.



# Abstracts of CURRENT ENDOCRINE LITERATURE

*Editor*; D. A. MCGINTY, *Collaborators*; A. R. ABARBANEL, F. N. ANDREWS, B. L. BAKER, F. A. DE LA BALZE, ISRAEL BRAM, R. A. CLEGHORN, RUCKER CLEVELAND, C. D. DAVIS, ANNA FORBES, M. B. GORDON, H. S. GUTERMAN, M. M. HOFFMAN, R. G. HOSKINS, C. D. KOCHAKIAN, H. S. KUPPERMAN, H. L. MASON, JANET W. MCARTHUR, THOMAS H. MCGAVACK, A. E. MEYER, K. E. PASCHKIS, A. B. PINTO, J. R. REFORZOMEMBRIVES, E. C. REIFENSTEIN, JR., G. G. RUDOLPH, L. T. SAMUELS.

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## PANCREAS

EDMONDSON, H. A., HELEN E. MARTIN, AND N. EVANS. Necrosis of renal papillae and acute pyelonephritis in diabetes mellitus. *Arch. Int. Med.* 79: 148 (1947).

Acute necrosis of the renal papillae was observed in 29 of 859 diabetic subjects in a series of 32,000 necropsies. Papillary necrosis is more common in women and is exceedingly rare in patients under 40 years of age as contrasted with pyelonephritis without necrosis in diabetic patients, among whom men predominate and occurrence under 40 is not unusual. The disease is highly fatal; only once was healing observed. It is most often part of acute pyelonephritis, which may be secondary to ascending infection of the urinary tract or to pyogenic foci elsewhere in the diabetic subject. Colibacilli and *Staphylococcus aureus* are the organisms most frequently isolated from acute pyelonephritis. The latter disease may be secondary to sepsis, which is an important cause of pyelonephritis in diabetic patients. Tuberculosis and actinomycosis were each noted once as the cause of papillary necrosis. The diabetic state, renal vascular disease (including Kimmelstiel-Wilson disease) and poor blood supply of the papillae are apparently contributing factors in the pathogenesis. In the same necropsy series, papillary necrosis was noted in 21 of 1,023 patients with pyelonephritis who were not diabetic; in 20 it occurred as a complication of urinary obstruction, which was most often due to disease of the prostate gland. Criteria for the diagnosis of papillary necrosis in diabetic patients cannot be rigidly defined but should include hematuria, renal colic, unexplained coma and sudden increase in severity of symptoms of known pyelonephritis. Retrograde pyelograms are usually diagnostic. Treatment should be directed toward preventing infection by early use of chemotherapy. After the onset of pyelonephritis, specific chemotherapeutic agents should be used in adequate amounts, as some patients may be saved.—(Authors' Summary and Conclusions)—I.B.

WHITE, PRISCILLA. Pregnancy complicating diabetes. *Pennsylvania M. J.* 50: 705 (1947).

When pregnancy complicates diabetes, five abnormalities may be revealed. These are maternal, obstetrical, chemical, fetal and placental in nature. In a series of 300 cases, the maternal abnormalities were vascular disease and hypo-ovarianism. The obstetrical abnormalities were an irritable uterus including early rupture of membranes with



uterus and human term placenta were made. The toxin activity was measured with the mouse assay method previously reported by the author. The mouse unit of toxin is defined as the minimum lethal dose for mice of 20 grams' body weight. The results confirmed the work of the author and others that tissue extracts, especially placental extracts, possess a toxin which has been regarded as a causative agent in toxemia of pregnancy. It was concluded that this toxin is thromboplastin, that its lethal effect depends on intravascular clotting and that antithromboplastin is the inactivator of the toxin. It is suggested, but not concluded, that thromboplastin may be the cause of toxemia of pregnancy.—*F.N.A.*

REYNOLDS, S. R. M. The relation of hydrostatic conditions in the uterus to the size and shape of the conceptus during pregnancy: a concept of uterine accommodation. *Anat. Rec.* 95: 283-296 (1946).

Accommodation of the rabbit uterus to the conceptus is divided into three phases: preparation by cellular proliferation, enlargement by hypertrophy and uterine stretching with enlargement of the fetus. The period of enlargement is characterized by spheroidal conceptuses. The fetus then elongates as uterine growth ceases at which time stretching of the uterine wall occurs. Since tension in a circular direction in a spheroid is less than in a longitudinal direction, accommodation to the conceptus during the period of stretching occurs by elongation because resistance to lengthening is one-half that to an increase in diameter. Maximum uterine growth is directly related to tension on the uterus exerted by the spheroidal conceptus whereas circulation of maternal blood is inversely related to this tension. Fetal death occurs most frequently at the end of the period of uterine enlargement and the rate of fetal growth is greatest during the period of stretching.—*B.L.B.*



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## BILATERAL FAMILIAL PHAEOCHROMOCYTOMATA, WITH PAROXYSMAL HYPERTENSION: SUCCESS- FUL SURGICAL REMOVAL OF TUMORS IN TWO CASES, WITH DISCUSSION OF CERTAIN DI- AGNOSTIC PROCEDURES AND PHYS- IOLOGICAL CONSIDERATIONS

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SUCCESSFUL surgical removal of a phaeochromocytoma is still a relatively rare accomplishment. A review of the available world's literature at this date has yielded reports of 176 such tumors, but only 47 of these were proven to be endocrinologically active by cessation of symptoms following surgical removal. The inherent difficulties in dealing with patients suffering from phaeochromocytomata are many. They may be divided into three categories and briefly listed as follows:

A. Diagnosis: Although the classical syndrome produced by such tumors is striking and clear cut (18), any individual case may lack one or more of the features which would ordinarily direct attention toward the correct diagnosis. Especially difficult are those patients in whom a permanent sustained hypertension is manifested and in whom the clinical diagnosis of essential hypertension may be made.<sup>1</sup>

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<sup>1</sup> Smithwick (7) found two such cases in the first 100 patients on whom he performed sympathetic ganglionectomy for hypertension. After removal of the adrenal medullary tumors which were disclosed by the operative exposure, both patients were cured of their hypertension.

B. Localization: When reasonable certainty of the presence of a phaeochromocytoma exists, localization of the offending tumor is often fraught with grave difficulties. Omitting tumors of the carotid body, which have not been known to produce hypertensive attacks, 89 per cent of all adult chromaffin tumors reported have been found in or adjacent to one or the other of the adrenal glands. Nevertheless, only when the tumor is of considerable size may it be positively identified in these areas by means other than surgical exploration.<sup>2</sup> Characteristic symptoms have occurred in patients subsequently proven to have chromaffin tumors of Zuckerkandl's body, the retroperitoneal ganglia, the semi-lunar ganglia (36) and even the thoracic sympathetic ganglia (30).

C. Treatment: Discovery of the site of the offending tumor still leaves the often complicated problem of its removal. Some tumors have been found so adherent to vital structures as to make removal impossible (6). In certain instances there have been wild fluctuations in blood pressure during the operation, and postoperative shock has often been extremely difficult to combat (2, 35).

With these exigencies incident to diagnosis, localization and operation, there is little wonder that surgical removal of such a tumor and postoperative convalescence has brought great satisfaction to the attending physicians. The fact that the few malignant phaeochromocytomata have but rarely been associated with hypertension (8) has yielded a further sense of security once the operation has been accomplished.

Recent experiences with two patients however, disclosed the fact that in certain instances more than one active tumor may be present. A woman and her niece had each previously been reported as cured by removal of a medullary adrenal tumor. Both had recurrences of symptoms, and each was found to have another tumor in the opposite adrenal. A description of the clinical course of these two patients, their responses to certain drugs, notably histamine, and a review of the literature on multiple tumors of this character form the basis of this report.

#### CASE REPORTS

*Case I. M.M. (#532786)*, a 22-year-old white female, entered the Johns Hopkins Hospital in April 19, 1946. She complained of recurrence of symptoms which had been relieved for a nine months' period by an operation performed four years previously at another hospital. Her mother had died with hypertension at the age of 28, having experienced symptoms quite similar to those of which the patient complained. *M.M.'s* past history included diphtheria, scarlet fever, ruptured appendix and a dislocated hip, all prior to adolescence. Six months before the onset of her present complaints there

<sup>2</sup> In expert hands perirenal air insufflation has proved helpful in localizing a tumor (6); but, in view of the dangers of the procedure and the difficulties of interpretation of the x-rays, it is not recommended as a routine diagnostic measure in these cases.

had been an illness of two weeks' duration, with headache and stiff neck, which she was told was poliomyelitis. There was apparently complete recovery. Menstrual periods had always been regular and the flow normal.

Four years before our observation began, the patient was seized suddenly, while in bed, with a violent generalized headache which lasted only 10 minutes. During the ensuing three months similar attacks recurred at approximately biweekly intervals. These episodes were accompanied by blanching and coldness of the extremities and were followed by flushing and a sensation of warmth, without conspicuous sweating. Because of these symptoms, she entered a hospital in New York where an operation was performed with removal of a pheochromocytoma from the left adrenal region. Studies performed and procedures carried out at that time were reported by Hyman and Mencher (19). The authors were aware that, at operation, the posterior layer of the capsule of the tumor was left in situ, because it was adherent to the surrounding structures.

Following operation the patient had no more attacks for a period of nine months, after which time she began to experience "brief periods of over-fatigue, complete lassitude, tremors, palpitations and alternate blanches and flushes. At first these spells were infrequent, but more recently have been daily occurrences. Within the past month I have had several headaches similar to those in the preoperative period, together with fainting spells and nausea. I am unable to feel completely healthy for even one entire day."<sup>3</sup>

At the time of admission the attacks were occurring several times a day. They commenced apparently spontaneously, although they were especially apt to occur when the patient was at stool or lying in bed on her left side. Heralded by severe generalized headache, they were climaxed by marked blanching and nausea and sometimes vomiting. Occasionally the patient became momentarily unconscious. After about three minutes she experienced great warmth, sweated and was left exhausted, the whole episode lasting about five minutes.

Physical examination in a resting state revealed a healthy appearing girl of 22. The skin was warm and slightly moist. Pupils were normal, and there were no visible abnormalities in the fundal vessels. The thyroid was diffusely and symmetrically enlarged to about twice average size. Neither thrill nor bruit was present. Heart rhythm was regular, rate slightly rapid. Although the apical impulse was accentuated, there were no evidences of cardiac enlargement. No significant murmurs were heard. Blood pressure was 120/80. The rest of the physical examination was normal.

During attacks she became markedly pale, especially in the extremities and around the mouth. "Goose pimples" might be apparent. An expression of great anxiety appeared, and on occasions she vomited. The blood pressure reached a maximum of approximately 200/150, but neither the cardiac nor the respiratory rate was much affected. Following the climax, she would become violently flushed, perspire freely and sink exhausted to the bed. The blood pressure did not return to normal for about 10 minutes.

Laboratory data: Serologic test for syphilis negative. Blood morphology normal. Urine contained neither sugar nor albumin; the sediment was normal. Concentration test, urea clearance and phthalein excretion were normal. Fasting concentration of the following was normal: whole blood sugar, non-protein nitrogen; serum cholesterol, calcium, inorganic phosphorus, alkaline phosphatase, chloride, bicarbonate, albumin,

<sup>3</sup> These quotations are from a letter written by the patient to one of the authors prior to her admission to the hospital.

globulin and potassium. An electrocardiogram revealed no abnormalities. An oral glucose tolerance test (Exton-Rose) was normal. X-rays of the skull, thorax and gall bladder were normal. Intravenous diodrast disclosed normal function and configuration of the urinary tract. The basal metabolic rate was minus 5 per cent.

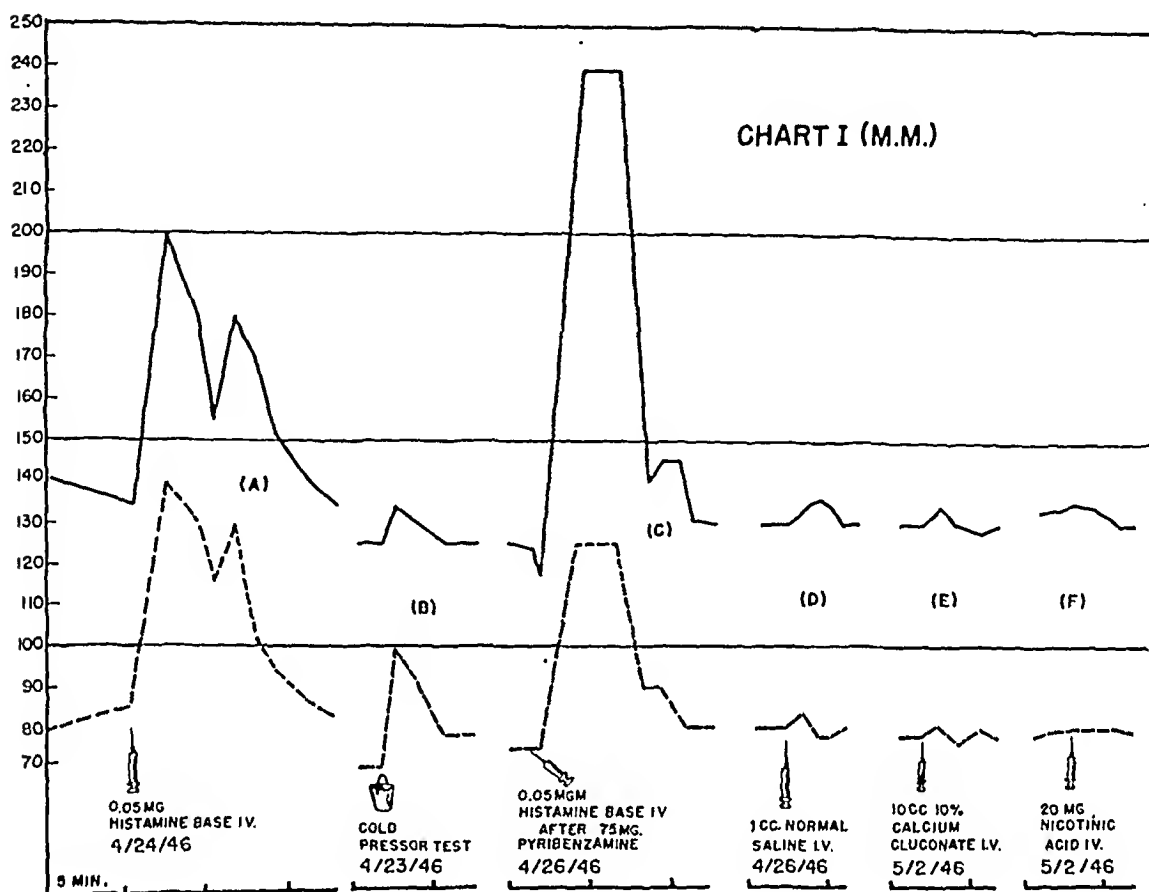
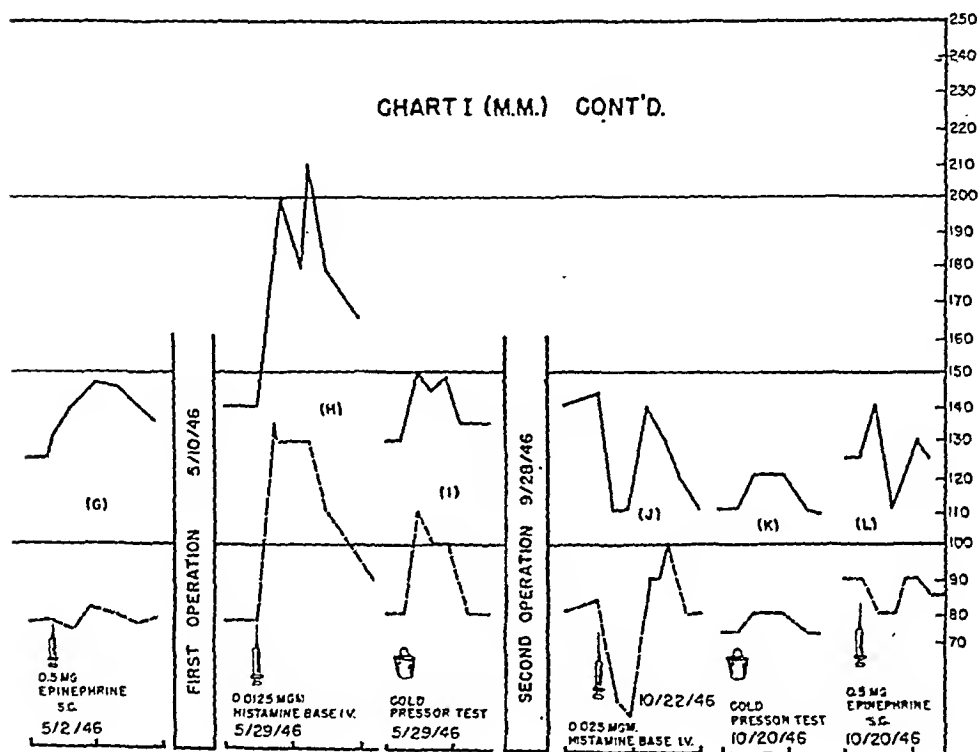


CHART I (M.M.). This chart shows the preoperative response of the systolic and diastolic blood pressure to the histamine and cold pressor tests, to the subcutaneous administration of epinephrine and to the intravenous administration of saline, calcium gluconate and nicotinic acid. The postoperative reaction to several of these tests is also depicted.

Special studies: Cold pressor test (17) produced a rise in blood pressure from 125/70 to 140/100 without symptoms (Chart I-b). Histamine test (32): 0.05 mg. histamine base in 1 cc. normal saline injected rapidly into the brachial vein was followed first by a momentary flush and then by an almost instantaneous rise in blood pressure from 140/80 to 200/140. This elevation in blood pressure was associated with a reproduction of all the symptoms and signs of the spontaneous attacks. Venapuncture performed while the blood pressure was receding was followed by a second lesser rise in blood pressure (Chart I-a). Repetition of the histamine test, 75 mg. of pyribenzamine having been given orally during the previous hour, resulted in an almost exact replica of the original histamine test (Chart I-c). Control injection of 1 cc. normal saline produced an insignificant blood pressure rise and no symptoms. Injection of 10 cc. of 10 per cent

calcium gluconate intravenously produced a marked generalized flush and slight fall in blood pressure. Injection of 20 mg. nicotinic acid in saline likewise produced a flush and a slight fall in blood pressure (Chart I). Administration of 0.5 mg. epinephrine subcutaneously produced a 20 point rise in systolic blood pressure and no subjective



response. Just prior to epinephrine injection, blood sugar was 76 mg. per cent, serum phosphorus 3.6 mg. per cent. Twenty minutes later the blood sugar was 76 mg. per cent, serum phosphorus 3.4 mg. per cent. Carotid sinus pressure was followed by a normal response. Massage of the flanks produced neither blood pressure change nor symptoms.

Operation: From the above it seemed likely that an excess of endocrinologically active chromaffin tissue was present. Since it was known that some capsular tissue had been left at the previous operation, it was decided with Dr. Colston that the left adrenal area should be explored.<sup>4</sup> At operation on May 10, 1946, a tumor the size of a golf ball was found in the left adrenal area, entirely encapsulated, so that complete removal was possible. During operation the blood pressure fluctuated wildly; high points of 240/145 seemed to coincide with manipulation of the tumor, normal blood pressure readings being obtained between paroxysms. Following ligation of all the vessels, the blood pressure no longer fluctuated but fell gradually. Approximately three hours after operation, the blood pressure fell below 80 systolic mm. mercury and 0.1 cc. epinephrine was in-

<sup>4</sup> A surgical report on these cases will be made separately by Dr. A. C. Colston who carried out all the operative procedures.

jected into the tubing of the continuous intravenous apparatus. A similar dose of epinephrine was given one hour later, and thereafter the blood pressure remained stable at 100/80. A litre of whole blood was administered during a 5-hour period after operation. Pathological examination of the tumor by Dr. Arnold R. Rich showed it to be a completely encapsulated benign pheochromocytoma.

At operation the pleura had been torn, and on the day after the operation signs of left pneumothorax and mediastinal emphysema were apparent. Cyanosis and respiratory difficulty which accompanied these complications were controlled by the administration of oxygen. The respiratory problems disappeared after the third day. At this time phlebitis of the right anterior tibial vein became evident at the site of the continuous intravenous injection.

On the tenth postoperative day the patient commented that she felt "woozy" when she lay on her left side. Since this position had evoked attacks before operation, blood pressure measurements were recorded with the patient in the left lateral decubitus. Upon assumption of this position, the blood pressure fell from 130/90 to 90/60, but 90 seconds later the patient exclaimed "Here it comes!" Blood pressure rose precipitously to 240/170; and the patient complained of nausea and headache, weeping pitifully because she was having another attack after a second supposedly successful operation. As the blood pressure reached its peak two minutes after the attack began, there developed flush and profuse sweat and the pulse rate rose to 120, remaining elevated for several hours after the pressure had returned to normal.

Spontaneous attacks of this sort continued to occur when the patient lay on her left side, and sometimes when she assumed the upright position. Intravenous histamine test was repeated (this time using only 0.0125 mg. histamine base), and a typical attack resulted, the blood pressure rising to 210/130 (Chart I-h). Immediately prior to this test, the blood sugar was 118 mg. per cent and serum phosphorus 4 mg. per cent. Because of the continuing attacks and the positive histamine test, the conclusion was reached that there existed another pheochromocytoma. The patient was discharged on June 1, 1946 and returned on September 23, 1946. Meanwhile the attacks had occurred less frequently but more severely than before.

Physical examination was the same as on the first admission. A spontaneous attack was observed on September 26 at 12:15 P.M. The blood pressure rose to 220/135. Fifteen minutes later the blood sugar was 116 mg. per cent, serum potassium 3.7 meq./litre. Serum potassium determination had been made in the fasting state both on this morning and on the previous one, the concentration having been 4.5 and 4.6 meq., respectively.

On September 26 the right adrenal area was explored by Dr. Colston. A tumor the size of a small lemon was found. There were three distinct nodules to the tumor, on the surface of two of which there was a thin film of yellow adrenal cortical tissue. The third nodule lay within fairly thick cortex, beyond which extended normal appearing adrenal tissue equivalent to about half a normal gland, which was not removed. The operation was very difficult, and there was much bleeding. The pleura was perforated, and the tumor capsule was ruptured with spilling of its contents into the wound. Efforts were made to retrieve these cellular contents, and the wound was washed with ether. There were three distinct rises in blood pressure during the operation, one when the 12th rib was being freed for resection and an instrument was passed beneath it, a second when the area was being palpated and the tumor was first felt and recognized, and a third when the tumor was mobilized for removal. The first two rises were relatively insignificant, but in the third the blood pressure reached 240/180, from which peak it gradually

fell when the pedicle was ligated. Glucose and saline (350 cc.) were given and the administration of 1000 cc. of whole blood was begun during the operation. Despite these measures the blood pressure fell to 85/50 four hours after operation. Six mg. of epinephrine chloride added to the continuous intravenous infusion over the course of the next five hours maintained the blood pressure at a normal level, and no further epinephrine was required. Pathologic examination revealed a rather "wild" looking and undifferentiated pheochromocytoma continuous with normal adrenal medulla and encased in a thick capsule. In a few areas tumor cells were seen within the blood vessels.

The ensuing three days were complicated by a fever of 103°, drowsiness amounting almost to coma and respiratory difficulty due to pneumothorax, which required tapping. Thereafter, aside from drainage of wound infection, recovery was uncomplicated. During later convalescence subcutaneous injection of 0.5 mg. epinephrine resulted in identical responses in blood pressure, blood sugar and serum phosphorus as recorded preoperatively. Cold pressor test was normal, a rise of 10 mm. mercury in both systolic and diastolic pressures being produced. Histamine test performed on October 12 produced a fall in blood pressure accompanied by flush and palpitation and followed by slight headache (Chart I-J). The symptoms were in no way similar to those previously obtained, and the test was judged negative. A letter from the patient on January 8, 1947 states that her wound is still draining but that she has been entirely free of all symptoms relating to the previous attacks and feels in excellent health.

*Case 2. Mrs. R.L. (#386001),* a 36-year-old white female, a maternal aunt of *Case 1*, entered the Johns Hopkins Hospital on May 19, 1946. Early in childhood a doctor told her of a heart murmur, but she recalls no episode suggestive of acute rheumatic fever. Between the ages of 16 and 18 years, she experienced some attacks of palpitation which, together with a moderate thyroid enlargement, resulted in a subtotal thyroidectomy. Ten days after the operation, she was seized with a sudden headache, the first of a long series of violent paroxysmal episodes. A description of a typical attack in order of appearance of symptoms follows: sudden onset of throbbing headache, sensation of hair standing on end, palpitation, aching in the legs and arms, nausea and a desire to eructate, profuse sweating over the entire body, and a sensation of pin pricks up and down the spine, with tingling of the tongue. These attacks would usually last about 20 minutes, frequently terminating in a violent sneeze. The patient felt very weak and exhausted for several hours after each attack.

Because of continuation and increasing frequency of these episodes, the patient entered a hospital in New York at the age of 27, nine years after their onset. A pheochromocytoma was removed from the left flank, the tumor being in the adrenal and adherent to the kidney. The entire left adrenal and left kidney were removed. Procedures incident to this hospital admission and operation have been reported in detail (1). The patient was free of attacks for approximately nine months after the operation. She has suffered with them ever since with the exception of complete freedom during her first pregnancy seven years before admission. During her second pregnancy four years before admission, however, the attacks were so severe that at the eighth month labor was induced and a healthy child was delivered manually. The patient was told at that time that her blood pressure was "over 200, and there was albumin in the urine."

During the year prior to her admission to this hospital, the attacks were more frequent and severe. She was also troubled by almost continuous sweating and "wave-like feelings of hot and cold." Her symptoms now assumed two distinct patterns, sometimes one merging into the other, but more often occurring separately. In type 1 there were "headache, blanching of the skin, pounding of the heart, goose-pimples and a sensation



of the hair standing on end." Such attacks were quite regularly induced by exertion and lasted only a few minutes. In type 2 she experienced a feeling of weight in the chest, pain in the episternal notch and down the left arm. Attacks of this sort might last throughout an entire day. Menses were quite regular and normal, and there had been no recent change in weight.

Physical examination showed a hyperkinetic woman who appeared to be in good health. Nutrition was normal, and there was no abnormal pigmentation. Examination of the optic fundi revealed narrowing of the fundal arteries and slight nicking of the veins at the crossings. There was no papilledema, and no exudates or hemorrhages were seen. The thyroid tissue beneath the scar was normal to palpation, and there were neither eye signs of Graves' disease nor tremor. There was no lymph node enlargement. The lungs were clear. The heart was overactive but not enlarged; sounds were vigorous, and there was present a rough systolic murmur, loudest at the apex, interpreted as of organic, probably rheumatic, origin. The right kidney was enlarged, readily palpable and not tender. The liver was at the costal margin; the spleen was not felt. Reflexes and peripheral pulses were normal. When the patient was examined while symptom free (except for the almost constant sweating), the blood pressure was highly variable, ranging from 120/80 to 250/140. The cardiac rhythm showed frequent serious changes of which the patient was quite unaware, seemingly unrelated to the blood pressure fluctuations. The rate might be found as low as 50 per minute, with abrupt changes to 140 or 150 per minute, and might be either regular or irregular.

During attacks the following observations were made: skin pallid and drenched, dyspnea with respiratory rate up to 50 per minute, extreme weakness and mental confusion, much like a patient coming out of an anesthetic. The blood pressure was as high as 280/160, but it should be noted that in such spontaneous attacks the blood pressure was always falling by the time the observer reached the patient, and it may well have reached higher levels.

The patient was discharged from the hospital after only four days, because her child became ill. She returned on September 30, 1946 with symptoms unchanged and physical findings identical. On both admissions she ran a slightly elevated evening temperature (100 to 100.4° per rectum) before operation.

Laboratory data: Serologic test for syphilis was negative. Blood morphology: no anemia; WBC ranged from 12,000 to 15,000 with normal differential count. Urine: highest concentration 1.028; albumin content varied from zero to moderate traces; glycosuria never present; sediment always normal. Fasting concentration of the following was normal: whole blood sugar, non-protein nitrogen; serum cholesterol, calcium, inorganic phosphorus, alkaline phosphatase, chloride, bicarbonate, albumin, globulin, potassium, and bilirubin. Cephalin flocculation was negative, and prothrombin time and serum amylase were normal. During a spontaneous attack, the blood sugar at 5 minutes was 118 mg. per cent; at 1 hour (when the blood pressure had just returned to 140/85) the sugar was 130 mg. per cent. Serum inorganic phosphorus taken at the same times was 4.6 and 4.7 mg. per 100 cc., respectively. Oral glucose tolerance test (100 Gm.): fasting 118 mg.,  $\frac{1}{2}$  hour 234 mg., 1 hour 290 mg., 2 hours 183 mg., 3 hours 60 mg., 4 hours 68 mg. per 100 cc. There was no glycosuria. Circulation time and venous pressure were normal. Phthalein excretion was 15 per cent in 15 minutes, 55 per cent total in 2 hours. Basal metabolic rate: plus 15 per cent; fair test; pulse 95.

Numerous electrocardiographic records were obtained, none of which were completely normal. The ST segment was consistently depressed in lead 2 and elevated in lead 3. T<sub>1</sub> was usually biphasic, T<sub>2</sub> sometimes inverted, and T<sub>4</sub> frequently inverted.

Although the rhythm was usually regular, on occasions, unassociated with clinical symptoms or the T wave changes noted above, it would become irregular. This consisted of periods of sinus arrest, lasting up to 4 seconds, during which there appeared nodal escape, and, in some instances, easily identified retrograde P waves following the ectopic nodal beat. The T waves were interpreted as being definitely abnormal, the sinus arrest and nodal escape beats as suggesting vagotonia to a marked degree. None of the electrocardiographic changes could be correlated with the occurrence of attacks of paroxysmal hypertension.

X-rays: Skull and sella normal. Teleoroentgenogram: normal. Intravenous diodrast: "The right kidney is well visualized, enlarged, a little low, but certainly shows nothing suspicious of a filling defect or a mass."

Special studies: Cold pressor test: the blood pressure rose from 135/90 to 160/105. Sodium amytal test: 3 doses 0.2 Gm. each given orally at  $\frac{1}{2}$  hour intervals. Blood pressure at the start was 250/130 (patient symptom free). Blood pressure fell steadily to 110/70 two hours after the last dose of amytal, at which time the patient was asleep. She awakened five hours after the last dose of amytal, at which time the blood pressure was 130/90. Histamine test: 0.05 mg. histamine base in 10 cc. saline injected rapidly by vein produced an immediate flush and coincident fall in blood pressure from 140/100 to 95/80. Within a minute the blood pressure had risen to 300+/190, the skin was pale, respirations were 90 per minute and the pulse 150 per minute (Chart II-a). The patient's symptoms were the same as during a severe spontaneous attack. Bending, lateral flexion and jumping produced no change in symptoms or blood pressure. Severe exercise, consisting of running upstairs, produced a severe attack but not the curious electrocardiographic pattern noted above. Subcutaneous injection of 0.5 mg. epinephrine was followed by no change in pulse, blood pressure (135/85), or symptoms over a half hour period.

Operation (10-4-46): It seemed probable that this patient, like her niece, had more physiologically active chromaffin tissue. Inasmuch as the left adrenal gland had already been completely removed, the right adrenal area was explored by Dr. Colston. A mass the size of a grapefruit was found, on the surface of which, and almost surrounding it, was plastered a paper-thin layer of yellowish brown tissue which was clearly adrenal cortex. It was impossible to remove the tumor and yet leave this tissue. Since the other adrenal had been removed at the operation nine years previously, it was feared that this might be her only remaining cortical tissue. The layer was so thin, however, that it was judged inadequate to support life. Some additional cortical tissue must be present. The mass was, therefore, removed intact. During the operation the blood pressure rose from 105/80 to 260/185, falling gradually to unobtainable levels as the operation ended. Response to transfusion was good, and during the succeeding 96 hours the systolic blood pressure fluctuated from 80 to 130 mm. mercury supported by periodic additions of epinephrine to the constant intravenous infusion. The total dosage of epinephrine administered during this period was approximately 50 mg. Fever reached 105° on the second night, at which time pneumothorax resulting from a pleural tear required tapping for fluid and air removal. The patient sweated profusely, appeared apathetic, dyspneic and cyanotic for several days. After the fourth day, the blood pressure required no further support by epinephrine; and convalescence thereafter was rapid despite wound infection and phlebitis at the site of the intravenous infusion.

Pathologic examination showed the mass to be a phaeochromocytoma surrounded by a thin layer of adrenal cortical tissue. The tumor cells were similar to those in the second tumor from M.M., but no tumor cells were found within the blood vessels.

Two weeks after operation there was no clinical or chemical evidence of adrenal insufficiency. Histamine test was repeated with normal results (Chart II-d). Cold pressor test and epinephrine response were also normal (Chart II-e and f). An oral glucose tolerance test on October 16, 1946 disclosed: fasting 88 mg.,  $\frac{1}{2}$  hour 118 mg., 1 hour 130 mg., 2 hours 80 mg., 3 hours 82 mg., 4 hours 80 mg. per 100 cc.

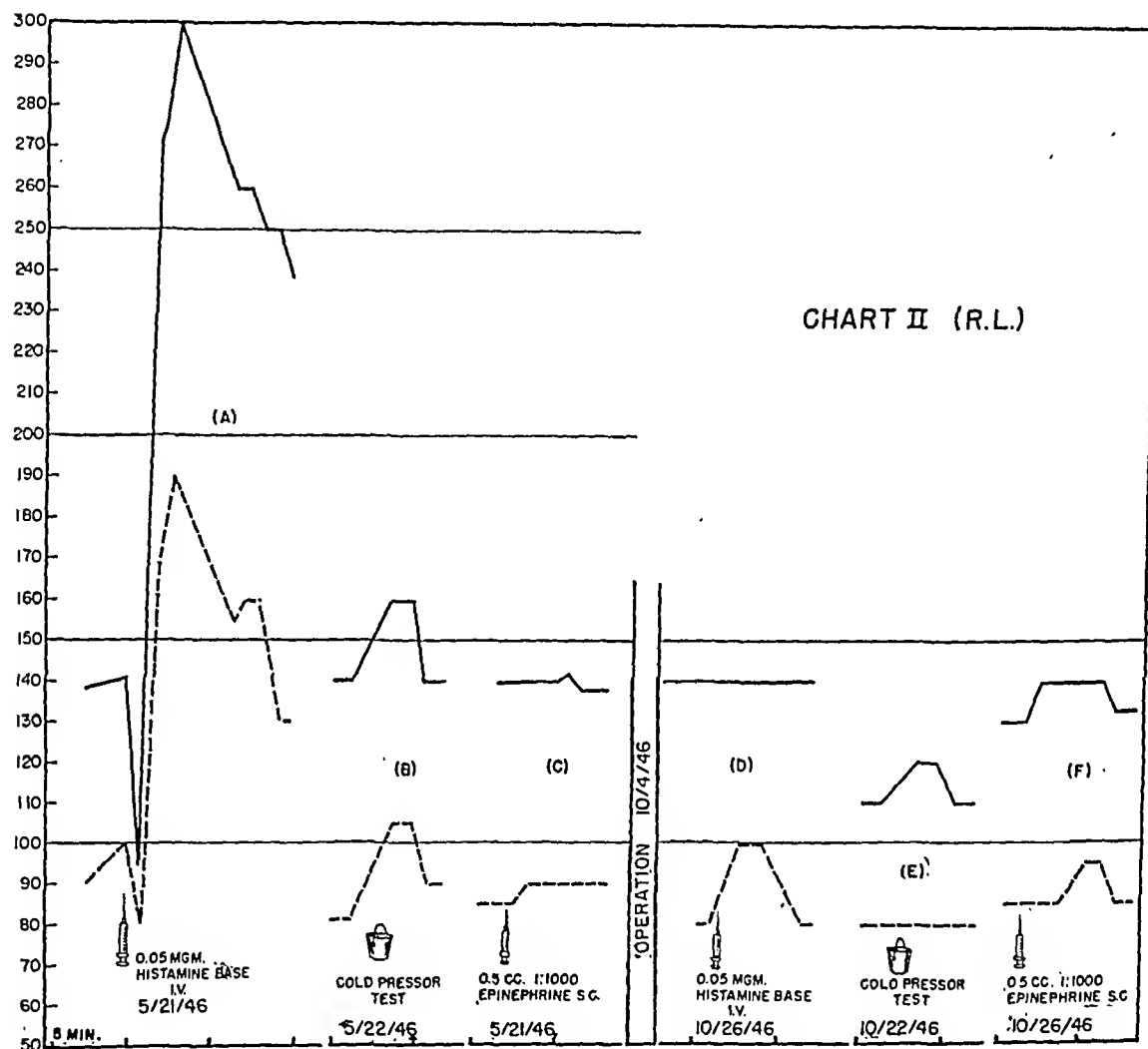


CHART II (R.L.). This chart shows the effect of the histamine and cold pressor tests and of the subcutaneous administration of epinephrine on the systolic and diastolic blood pressure before and after operation.

A letter from the patient dated February 6, 1947 states: "Anything resembling previous attacks has entirely disappeared. My blood pressure is 120/80; there is absolutely no sweating and I am quite strong. My circulation does not appear to be normal, however. My hands blanch from the palms to the finger tips, and at times the digits remain numb long enough to be painful. The tip of my nose is usually cold."<sup>5</sup>

<sup>5</sup> These symptoms of Raynaud's phenomena had been present since the age of 18 and had not been materially altered by her first operation (1).

Review of the literature reveals (see Appendix for details) 15 cases of bilateral phaeochromocytomata in which the tumors were located in or near the adrenal glands, and two cases each with two tumors arising in Zuckerkandl's body and the retroperitoneal ganglia.

It should be emphasized that no surgical attempt was made to remove the tumors in any of these cases. There is no proof, therefore, that the phaeochromocytomata were actively secretory and responsible for any of the patients' symptoms, though in many instances it seems altogether likely. Insofar as we are aware, the patients here reported are the first from whom more than one phaeochromocytoma has been removed. MacKenzie, in discussing a paper by Hyman and Mencher (19) mentions a patient of his own whose paroxysmal hypertension disappeared after removal of a tumor. Four and a half years later the symptoms recurred. Though careful exploration of both adrenal areas failed to reveal a tumor, MacKenzie was of the opinion that another phaeochromocytoma existed somewhere. It would seem that certain individuals have a propensity for tumefaction of adult chromaffin tissue. This propensity might result from an increased stimulus to such tissue or from a hyper-responsiveness of the chromaffin tissues to a normal stimulus. With the above experiences in mind, there was considerable mental reservation in our answers to the question posed by both patients: "How do you know we won't develop still more tumors?"

In this same connection another unique feature of the cases here reported was their close relationship. The familial incidence is even more impressive since the mother of the younger patient, who was also the sister of the elder patient, seems almost certainly to have died of the same condition. It is interesting to note that there is also a high incidence of thyroid disease in this family; *R.L.*, her two sisters and her mother were all subjected to thyroidectomy in their youth.

The association of "thyroid disease" and phaeochromocytomata is commonly found in the literature of the latter condition. Usually the anatomical status of the thyroid has been noted as simply diffuse adenomatous enlargement. In many, a diagnosis of hyperthyroidism (36, 38) has been made because of elevated basal metabolic rates and suggestive symptomatology. It is difficult to discern from reported cases which of several possibilities provides the most suitable explanation for this association. True hyperthyroidism may have been present and purely coincidental. The incidence seems far too great to make this likely. There may be some direct action on the thyroid by the secretion of the phaeochromocytomata, which induces non-secretory thyroid enlargement, perhaps through its vascular effects. Diagnostic difficulty is further enhanced by the nervousness, tremor and hypermetabolism produced by epinephrine.

We have been impressed by the value of histamine as a method of inducing attacks in patients with phaeochromocytomata. The use of histamine as a diagnostic test was first suggested by Roth and Kvale (32). These investigators compared the effect of intravenous injection of 0.05 mg. histamine base with that of the cold pressor test in a series of normal individuals, hyper-reactors (to the cold pressor test), patients with well established hypertension and in three patients with phaeochromocytomata subsequently demonstrated at operation. They found that "in the first three groups the blood pressure rose to a level somewhat less than the elevation obtained by the cold pressor test." In the three patients who suffered from phaeochromocytomata, the blood pressure "rose approximately 100 mm. more than the elevation obtained by the cold pressor test, and was accompanied by the characteristic symptoms of a typical spontaneous attack."

Our experience with the use of the Roth-Kvale test coincides closely with theirs. In our patients the injection of histamine produced an abrupt rise in blood pressure which dwarfed that which accompanied the cold pressor test (see Charts). The drug seemingly induced episodes which were in all respects identical with spontaneous attacks. The test appears to have a two-fold value, first as a method of producing attacks at will for study, and, second, as an indication of the presence of phaeochromocytoma. In the patients here reported, so long as the test remained positive, it was possible to find and remove more tumors. Conversely, so far as known, no phaeochromocytomata have been found in patients in whom the test was negative.

The mode of action of histamine in producing these attacks is not clear. Its peripheral vasodilating effect is probably not responsible, since the intravenous injection of neither calcium nor nicotinic acid produced attacks despite evidence of pronounced peripheral vasodilatation.<sup>6</sup> Our attempts to block the effect of histamine by the use of pyribenzamine orally were unsuccessful, though the dosage of pyribenzamine used (75 mg.) may have been insufficient for the purpose.

It has long been known that the intravenous injection of histamine into normal animals is followed by a marked increase in epinephrine discharge, a concurrent rise in blood pressure preceded by a transitory fall, and a parallel hyperglycemia (12, 4, 37). That these effects are due to stimulation of the adrenals is apparent from the demonstration by Dale and others that they do not occur following removal of these glands (4, 37, 21). Whether the motivation of histamine action is directly on the medullary

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<sup>6</sup> Histamine may have produced vasodilatation of internal organs, whereas the other two drugs may not have.

cells or is mediated through some reflex mechanism<sup>7</sup> is not clear, owing to conflicting experimental observations (12, 4, 37, 21).

Whatever the mechanism, it is possible that histamine calls forth a maximum response from all active chromaffin tissue. Thus a patient with a phaeochromocytoma would have more tissue available for such a response. Attacks of hypertension in patients with phaeochromocytomata have followed injection of other drugs. Mecholyl was immediately followed by an attack in Mayock's patient (26). Although in the majority of cases subcutaneous injection of epinephrine has produced negligible symptoms, in an unreported case observed by Schoenbach (33), injection of small doses repeatedly induced typical paroxysms.

Thus far we have seen no ill effects from the use of the Roth-Kvale test. Terrific attacks of paroxysmal hypertension so induced have caused alarm, yet in our patients, attacks induced by histamine were no more severe than some spontaneous attacks, and it seems to us unlikely that the dangers inherent in the test would be more severe than would inevitably be suffered by the patient in subsequent spontaneous episodes. If the above philosophy is accepted, and there is at present to our knowledge no contradictory evidence, the dangers inherent in the test may have been overstressed in a recent discussion by Burrage (5).

It may be that, in patients suffering with sustained hypertension resulting from phaeochromocytoma, histamine may not induce a conspicuous further rise in blood pressure owing to already near maximal narrowing of the arteriolar bed. In cases in which the arteriolar narrowing has not become anatomically fixed, the use of epinephrine antagonists such as those of the Fournau series (20), may prove of great diagnostic aid.

Passing comment should be included on one further feature, namely, the wide variation in the effect of medullary adrenal tumors on carbohydrate metabolism. Some tumors invoke marked glycogenolysis with each paroxysm of hypertension, as would be expected from a massive injection of epinephrine. In one instance (18) the clinical syndrome of diabetes was present, which entirely disappeared after removal of the tumor and has remained absent to date, nearly five years later. Other individuals with phaeochromocytoma; however, show little or no effect on their carbohydrate metabolism. The two patients here reported appear to be of the latter group. At no time did either patient manifest glycosuria; glucose tolerance

<sup>7</sup> This reflex may be initiated by a rapid fall in blood pressure. Very transitory fall in blood pressure was noted coincident with histamine injection in our patients, and in one instance (*M.M.*) a momentary fall in blood pressure was observed preceding a spontaneous attack. Wada (37) noted this transitory hypotension following injection of histamine into his dogs. He further observed that the longer it lasted, the greater the subsequent epinephrine discharge.

tests were essentially normal and the concentration of blood sugar and serum inorganic phosphorus showed little change after either spontaneous or induced attacks. The only suggested explanation has been that some tumors may secrete an epinephrine-like pressor hormone, rather than epinephrine itself (18). Evidence against this hypothesis is the fact that 0.5 mg. epinephrine subcutaneously did not produce evidence of glycogenolysis in our patients either before or after operation.

### SUMMARY AND CONCLUSIONS

This report contains observations on two patients, members of the same family, each of whom had phaeochromocytomata of both adrenals. Successful surgical removal of three tumors from these two patients with eventual relief of symptoms is described.

The histamine test, devised by Roth and Kvale, proved to be a valuable aid to our diagnostic confidence in the existence of the tumors. In the three instances here reported of recurrence of symptoms after removal of a tumor, this test proved a reliable guide for further exploratory surgery.

Previous reports of multiple phaeochromocytomata are reviewed.

The importance of diagnosis of the presence of phaeochromocytoma cannot be overemphasized, as this is one of the few causes of hypertension which can be successfully attacked at the present time. Since phaeochromocytomata may present the symptomatology of paroxysmal hypertension, benign hypertension, malignant hypertension or hyperthyroidism, it is urged that the clinical use of histamine and other pharmacologic tests become more widespread.

### APPENDIX

We have been able to find in the literature reports of 17 cases of multiple phaeochromocytomata; 15 of these had tumors situated in or near the adrenal glands. Four of these, mentioned by Buchner (3), Lascagna (24), Gorog (14) and Marchetti (25), are pathological reports only and contain no clinical material. The first two of these were malignant. Of the entire series, three others also suffered from malignant tumors.<sup>8</sup> Eisenberg and Wallerstein (11) report a woman of 63 who entered the hospital because of a mass in the neck. Her blood pressure had ranged between 120/75 and 130/85, and she had suffered no symptoms suggestive of paroxysmal hypertension. She eventually died of what was found to be massive multiple metastases from bilateral malignant phaeochromocytomata. She had also a papillary adenocarcinoma of the thyroid, which had caused the chief complaint. Gravier and Bernheim (15) reported a patient whose age and sex are not given but whose blood pressure was recorded as 105/50. This patient died of mediastinal metastases from bilateral malignant phaeochromocytomata. Scarcely more information is given about King's (22) patient, a 30-year-old man who died of

<sup>8</sup> Certain reservations must be made in considering these five malignant cases truly bilateral. Although in each case the tumors on the two sides were allegedly independent, there remains a real possibility that one was a metastasis from the other.

hemorrhage following tooth extraction. No blood pressure is recorded. It is interesting to note that his thyroid gland had been removed 14 years previously. Pathological examination revealed bilateral malignant phaeochromocytomata with metastatic nodules in the adrenal, liver, skin and bowel. None of these three patients is known to have experienced symptoms of paroxysmal hypertension.

Of the 15 patients with bilateral tumors, seven were known to have suffered from constant hypertension. In all seven the tumors were benign. Biebl and Wichels (38) described a 36-year-old male diabetic who died of what was subsequently proven to be a cerebral hemorrhage. He maintained a blood pressure of 208/120 to 225/125 and suffered from a similar, although less severe, vascular accident two years previously. At autopsy bilateral retroperitoneal phaeochromocytomata were found. There were no metastases. Herde's (16) case report contains no clinical information except that the blood pressure was known to be "elevated." The patient, a 45-year-old woman, died of a cerebral thrombosis and pulmonary edema. Bilateral benign phaeochromocytomata were found at postmortem examination. A 14-year-old girl was reported by Kremer (23). She suffered from what appeared to be typical malignant hypertension, together with a severe endocrinopathy characterized by obesity, polycythemia and masculinization, with hypertrichosis and underdevelopment of the breasts and genitalia. At autopsy she was found to have bilateral ovarian cystomata as well as bilateral benign phaeochromocytomata.

Paul (29) quotes Kulschera and Aichberger as having seen a 72-year-old female with a systolic blood pressure ranging from 185 to 192 mm. Hg. She suffered from periodic attacks of headache and dizziness, eventually dying of cardiac insufficiency. Pathological examination disclosed, in addition to chronic nephritis and cardiac hypertrophy, benign bilateral phaeochromocytomata. Popken's (31) patient, a 43-year-old woman with known hypertension, died from a cerebral hemorrhage. At autopsy benign phaeochromocytomata were found in both adrenal glands. Schroeder (34) reported a 42-year-old woman with diabetes which was resistant to insulin. She had a five year history of hypertension, recently reaching a maximum of 220/140, and finally died of bronchopneumonia and diabetic coma. A small calcified tumor was found in the thyroid gland at autopsy, in addition to bilateral benign phaeochromocytomata.

Frankel (13), in 1886, reported an 18-year-old girl with known hypertension, although no actual blood pressure measurements are mentioned. For the previous four years she had been suffering from episodes of what closely resembled paroxysmal hypertension. These consisted of palpitations, headache, feeble rapid pulse, hyperpnea, pain in the chest, nausea and sometimes vomiting. Death occurred in one of these attacks. Bilateral adrenal tumors found at autopsy gave a positive chrome reaction. Although they were called angiosarcomata at the time, Edward (10) states that they were almost certainly phaeochromocytomata. Only one other patient, reported by Paul (29) had a history suggestive of paroxysmal hypertension. After induction of cocaine anesthesia, he had a sudden attack of headache, sweating, dyspnea, tachycardia and dilation of the pupils, and died two hours later. It is not known whether he had experienced similar episodes previously, and no blood pressure was taken. At postmortem examination, bilateral chromaffin tumors were found, the extract of which was experimentally shown to have a pressor effect.

Of these 15 cases of bilateral phaeochromocytomata, therefore, one-third were malignant. Although clinical information is far from complete, none of these patients is known to have suffered from either constant or paroxysmal hypertension. Of the ten reports of patients with benign tumors, seven contained information as to the blood pres-



sure. All these patients had hypertension. Only two patients had symptoms clearly suggesting paroxysmal hypertension. Although this tendency for patients with malignant phaeochromocytoma to have normal blood pressure agrees with the observations of McGavaek (27), it must be emphasized that this is by no means infallible. There are a number of reports in the recent literature of hypertension in patients with malignant phaeochromocytomata.

In addition to these cases of bilateral phaeochromocytomata, all of which were located in or near the adrenal glands, there are several additional reports of interest. Cragg (8) published a pathological report on a 39-year-old female who died of bronchopneumonia. At autopsy she was found to have a large malignant tumor of the carotid body plus bilateral benign tumors of the bodies of Zuckerkandl. McCullagh and Engel (28) reported another case of multiple phaeochromocytomata. The patient, a 28-year-old man, was a known diabetic with persistent hypertension. In addition he had an elevated basal metabolism and symptoms which led to the diagnosis of hyperthyroidism. Thyroidectomy was performed, but he developed a furuncle postoperatively and died of cellulitis. At autopsy he was found to have a phaeochromocytoma of the right adrenal gland plus another tumor, smaller but similar grossly and microscopically, of Zuckerkandl's body.

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# SYNDROME PRODUCED BY ABSENCE OF THE GERMINAL EPITHELIUM WITHOUT IMPAIRMENT OF THE SERTOLI OR LEYDIG CELLS

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THE classification of abnormalities of the endocrine and spermatogenic functions of the testes is improving. Until a few years ago, the subject was extremely confused, largely as a result of the inadequacy of the methods of study. Histological studies of testicular biopsies and assays of urine for various hormones has made it possible to isolate several syndromes in which the testicular pathology is the primary abnormality.

The present paper gives the clinical, anatomical, and biological characteristics of a new testicular syndrome. Evidence of the existence of a second testicular hormone is strengthened by the observations reported and a specific function for the Sertoli cells is established.

Before discussing this new syndrome, it will be helpful to mention briefly three related syndromes.

1. In 1942 Klinefelter, Reifenstein, and Albright (16) (see Table I) described a syndrome characterized by gynecomastia, aspermatogenesis without aleydigism, and increased excretion of follicle-stimulating hormone (to be designated hereafter as F.S.H.) They presented nine cases. Testicular biopsies showed hyalinization of all, or nearly all, of the tubules. The pathologic process involved the germinal cells as well as the cells of Sertoli. There appeared to be an increase in the number of Leydig cells but this appearance was probably accounted for by the shrinkage of the tubules. The testes were small and soft and the patients, of course, were sterile inasmuch as the semen showed azoospermia. F.S.H. was excreted in the urine in excessive amounts in all the patients and to a degree comparable to that found in castrates. All patients presented bilateral gynecomastia. The estrin excretion was studied in two cases and found to be within normal limits.

2. In 1941 Albright et al. (1) described a group of patients with eunuchoidism and increased F.S.H. excretion (Table No. I, Column 2). Heller, Nelson and Roth (11) studied a group of these patients. Clinically, they

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presented manifest sexual infantilism of the type which is ordinarily associated with prepuberal lack of Leydig cell function, and biopsies of the scrotal content revealed no actively functioning testicular tissue. All cases presented marked elevation of the F.S.H. excretion in the urine.

TABLE I

TABULATION OF THE DIFFERENCES BETWEEN THE KLINEFELTER, REIFENSTEIN AND ALBRIGHT SYNDROME (1942), THE EUNUCHOIDISM WITH INCREASED F.S.H. EXCRETION, THE CASTRATION SYNDROME, AND SYNDROME HERE DESCRIBED

	No. 1 Klinefelter, Reifenstein, and Albright (1942)	No. 2 Eunuchoidism With Increased F.S.H.	No. 3 Castrates	No. 4 Syndrome Here Presented
Clinical manifestations of Hypogonadism	absent or scarce	present	present	absent
Gynecomastia	present	frequently present	frequently present	absent
17-ketosteroids	normal or subnormal	reduced	reduced (usually)	reduced
F.S.H.	increased	increased	increased	normal
Azoospermia	present	present	present	present
Sertoli cells	destroyed	absent or atrophied	absent	present
Germinal epithelium	intense lesion	absent or atrophied	absent	absent
Walls of the tubules	hyalinized	absent or atrophied	absent	normal
Leydig cells	apparent hyperplasia	absent	absent	apparent hyperplasia
Testis size	small	absent or atrophied	absent	small

3. The third syndrome (See Table I, Column 3) is that produced by bilateral castration (surgical, traumatic, or infectious). The clinical picture is that characteristic of gonadism with certain differences depending on

the age at which the castration occurred. The F.S.H. in the urine is excreted in increased amounts (Smith (21); Albright et al. (1); Catchpole, Hamilton, and Hubert (3)); the 17-ketosteroid excretion is usually decreased (Albright et al. (1)).

The five patients who are the subject of this paper fall into a syndrome characterized by the eight following criteria (See Table I, Column 4).

1. bilateral small testes, comparable in size to the testes of prepuberal boys,
2. in contrast to the small testes, a phallus normal in size and appearance, and scrotum, perineal hair, and other secondary sexual characteristics normal in appearance,
3. the general physical status in every way normal,
4. sterility, with azoospermia,
5. a past history negative for diseases which could have been harmful to the germinal epithelium,
6. a testicular biopsy which shows (Figs. 1, 2 and 3) (a) small, seminiferous tubules, (b) complete absence of germinal epithelium, (c) presence of Sertoli cells and normal basement membranes of the tubules, and (d) normally appearing Leydig cells, and
7. a normal F.S.H. titer in the urine and a reduction of 17-ketosteroid excretion.

#### MATERIAL AND METHODS

This work is based on a study of five patients, all of whom complained of primary sterility. Their ages varied from 22 to 36 years.

##### *Methods:*

1. Examination of the testes:
  - (a) physical examination,
  - (b) examination of semen,
  - (c) testicular biopsy; the testicular tissue was fixed in Bouin's solution, imbedded in paraffin, and stained with hematoxylin-eosin or Masson's stain.
2. Hormonal studies:
  - (a) assay of F.S.H. in the urine. The method of Zondek was used with the modifications introduced by Klinefelter, Albright and Griswold (15). The normal value for the adult male is not less than 6 and not more than 52 mouse units per 24 hours.
  - (b) Measurement of the urinary 17-ketosteroids. The method of Callow, Callow, Emmens and Stroud (2) was utilized for hydrolysis, extraction, and isolation; the method of Zimmerman was used for colorimetric assay. Normal values for our adult male population vary between 12 and 18 milligrams per 24 hours.

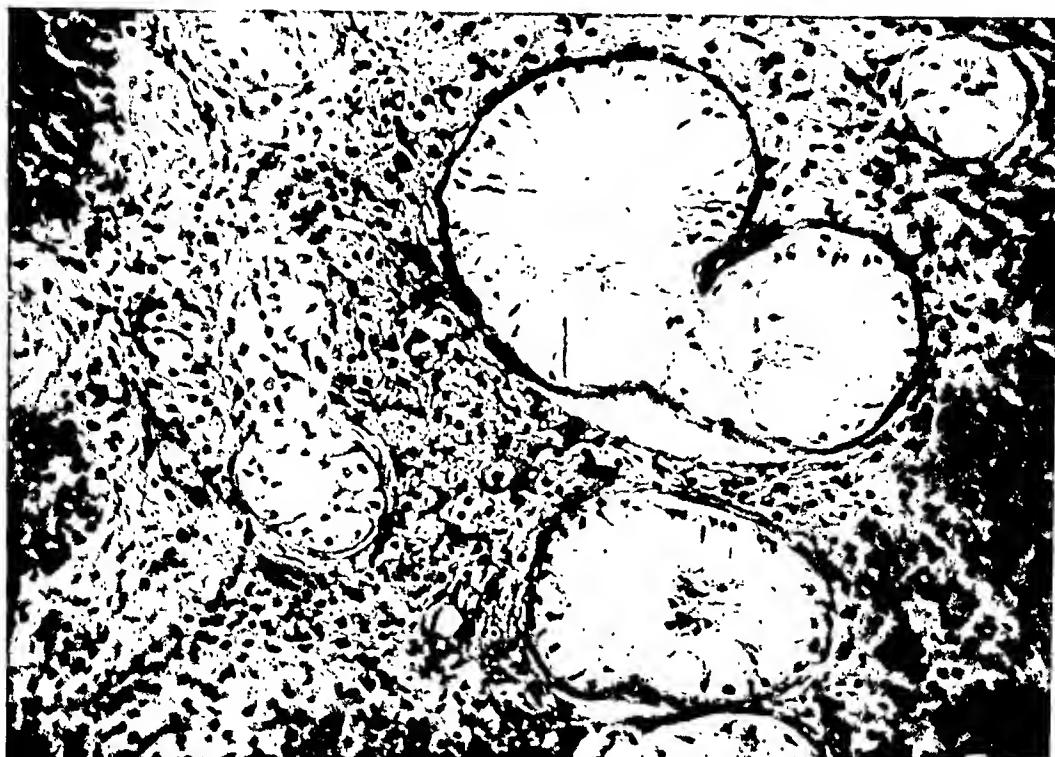


FIG. 1. Testicular Biopsy. (Low Magnification.)

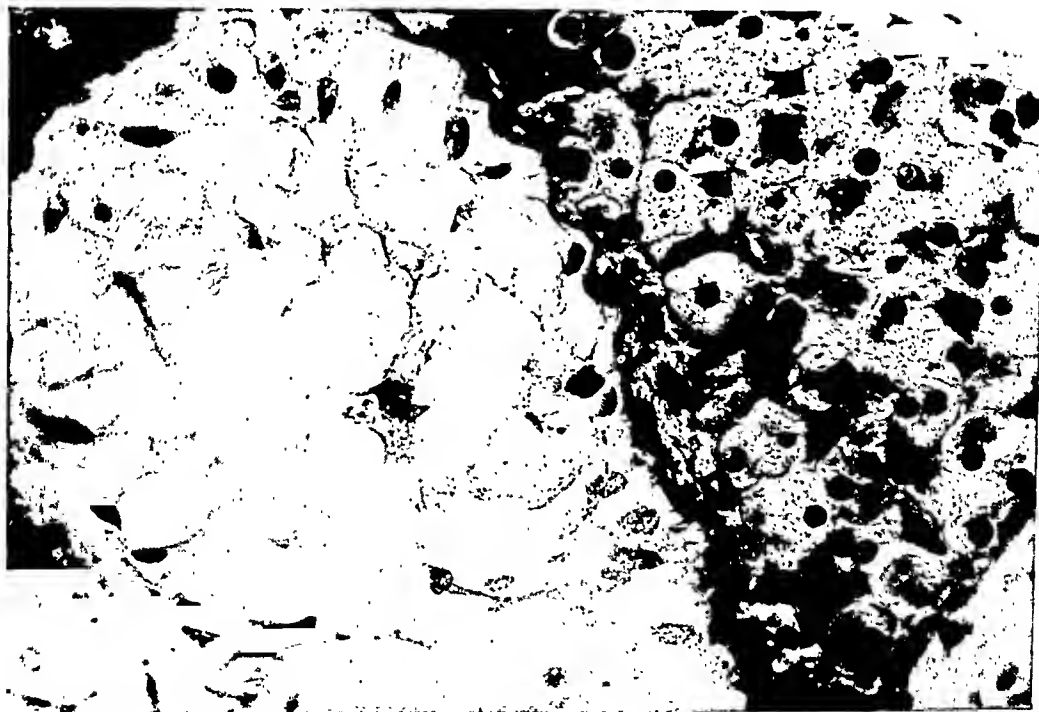


FIG. 2. Testicular Biopsy. (High Magnification.)

## RESULTS

The personal histories did not disclose any infectious diseases, surgical procedures, traumata, intoxications, faulty diets, x-ray irradiation, et cetera, which could have been harmful to the germinal epithelium. There was no history of cryptorchidism or delayed descent of the testes. There was nothing to suggest abnormal adrenal cortical function. The libido, erections, and ability to have sexual intercourse were, in each instance, normal. The routine laboratory tests were entirely normal.

In each case both testes were normal in respect to shape, position, sensibility and orientation, but were decreased in size. They measured



FIG. 3. Testicular Biopsy. (High Magnification.)

approximately  $3 \times 2 \times 2$  cm. The epididymus, prostate, and seminal vesicles and the penis and scrotum were normal in size and shape.

No spermatozoa were ever found in the semen despite repeated examination. The analyses done after centrifugation were also negative, and the sediment showed neither normal nor abnormal germinal cells.

The testicular biopsies showed (Figs. 1, 2 and 3) the seminiferous tubules to be markedly decreased in size and devoid of spermatogenic cells. Even the basal spermatogonia were absent. The walls of the tubules showed an apparent thickening, and the cells of the walls had nuclei elongated and not pyknotic. Inside the seminiferous tubules only the cells of Sertoli could be



found. The structure of their protoplasm was characterized by a fine reticulum which made us think that they were still active. The nuclei were elongated, oval, or triangular in shape, their structure showing activity as judged by the fact that the chromatin, even if slightly diffuse was perfectly visible and not conglomerate. A rounded nucleolus with white center was located asymmetrically inside each nucleus. There was the appearance of an hyperplasia of the Leydig cells which was probably due to the reduced size of the tubules as a result of the lack of germinal epithelium. The protoplasm of the Leydig cells appeared to be active as did the nuclei. The lesions were entirely intratubular and, in agreement with Charny

TABLE II  
RESULTS OF THE F.S.H. AND 17-KETOSTEROIDS ASSAYS  
(F.S.H. in Mouse Units)

Patient	F.S.H. 6	F.S.H. 12	F.S.H. 24	F.S.H. 48	F.S.H. 72	F.S.H. 96	17-K's.
No. 1				pos.	neg.	neg.	
No. 2				pos.		neg.	
No. 3		neg.	neg.	neg.		neg.	5.54 mg.
No. 4	pos.			neg.	neg.	neg.	9.74 mg.
No. 5	pos.		neg.	neg.		neg.	8.15 mg.

and Meranze (4), it can be said that the lesions did not appear to be degenerative or inflammatory; thus, there was no necrosis or desquamation of the tubular epithelium and there was absence of peritubular fibrosis.

The results of assays of the pituitary follicle-stimulating hormone (F.S.H.) in the urine are presented in Table II, where the amounts are expressed in mouse units per 24 hours. It will be seen that all five patients excreted less than 96 mouse units per 24 hours. In two patients the test was carried out for 72 mouse units, and in both instances the results were negative. Two of the five patients were positive for 48 mouse units per 24 hours. Only two patients were tested for 6 mouse units per 24 hours, and both were positive. In summary it can be said that the patients presented normal levels of F.S.H.

Urinary assays for 17-ketosteroids were carried out on patients 3, 4, and 5 (See Table II). The amounts excreted were slightly subnormal.

## PATHOGENESIS

As to the cause of the absence of the germinal epithelium, one of us (Trabucco (21)) attributed it to a failure of penetration of the primary gonocyte into the embryo during the presomitic or parasomitic period. In this connection there are some well known pertinent facts. The testicular tissues have two origins. The Sertoli and Leydig cells and the connective tissue cells come from the embryonic mesenchyma; these cells give shape, consistency and sensory innervation to the testes and constitute the framework of the seminiferous tubules. The germinal cells have an independent origin. We believe that the absence of the germinal epithelium is a result of lack of migration of the primary gonocytes into their definitive place on the urogenital ridge. Such a condition has been produced experimentally in animals.

On the other hand, as a cause for the absence of germinal epithelium, we cannot exclude the possibility that it is related to some embryological or post-natal nutritional failure (avitaminosis E, et cetera), to some endocrine factor, or to some abnormality of the steroid metabolism. It has been shown (Masson (18)) that delta 5 pregnenolone, isolated from pig's testes, is able to prevent the atrophy of a rat's testes which occurs after hypophysectomy. Furthermore, under appropriate circumstances, testosterone is able to stimulate the germinal epithelium. Unfortunately, the effect of testosterone on human seminiferous tissue has not been sufficiently studied. Although the histological picture of the testis (Figs. 1, 2 and 3) looks very similar to that which Charny and Meranze calls "complete atrophy," we prefer to speak of it as "absence of the germinal epithelium."

## DISCUSSION

Greep, Van Dyke, and Chow (9) showed that F.S.H. from the hog pituitary has a specific action on the seminiferous tubules of male rats. Thus, hypophysectomized male animals treated with F.S.H. show an increase in testicular weight with an associated atrophy of the sex organs. The testes, on histological examination, show stimulation of the seminiferous tubules and atrophy of the interstitial cells. It would appear, therefore, that F.S.H. is the pituitary gametogenic hormone. Furthermore, it has been shown in rats (Engle (7), Evans and Simpson (8)), in mice (Martin (17)), in guinea pigs (Severinghaus (19)), and in horses (Hellbaun (11)) that, following castration, the gonadotropic content and, in some instances, the secretory activity of the pituitary increases. This has been interpreted as being due to withdrawal of one or more testicular hormones. It is generally accepted that the increased urinary F.S.H. excretion which appears in a male castrate reflects the augmented function of the pituitary gland that results when gonadal function is absent (Hamburger (10), Smith (20)).

It has not yet been established which testicular element is responsible for inhibiting F.S.H. In order to throw further light on this question, we review the F.S.H. excretions in three testicular syndromes in which a clear-cut histological picture for the testicular tissue has been demonstrated. In syndromes #1, #2, and #3 (See Table II) the increased amount of F.S.H. is associated not only with absence of the germinal epithelium, but also with absence, or at least definite lesions, of the Sertoli cells. In the group of patients here presented, in which F.S.H. was excreted in normal amounts (See Table II), the germinal epithelium was absent but the Sertoli cells were present and normal in appearance (See Table I) (See Figs. 1, 2 and 3). This strongly suggests that the Sertoli cells inhibit the production and excretion of F.S.H.

It could be argued that the F.S.H. excretion is normal in the syndrome under discussion because the Leydig cells are normal; it must be remembered, however, that in the syndrome described by Klinefelter, Reifenstein and Albright (16) the F.S.H. excretion is increased in spite of normal quantities of Leydig cells and normal amounts of 17-ketosteroid in the urine. We feel justified in assuming that neither the histological studies of the Leydig cells nor the amounts of urinary 17-ketosteroids allow one to predict how great the amount of urinary F.S.H. will be. On the other hand, we should like to emphasize that, in the syndrome here presented, where the amount of F.S.H. present in the urine is normal, the Sertoli cells are normal while the germinal epithelium is absent. We conclude that there is a great probability that the Sertoli cells regulate gonadotropic activity of the pituitary glands through a product of their secretions.

All our patients were adults. This brings up the question as to why the Sertoli cells had not disappeared or had not altered in morphology since they lacked their normal nutritional or supportive function. Indeed, the protoplasm and nuclei of the Sertoli cells looked decidedly active. It seems logical to assume that this behavior was the result of some other function of these cells and we suggest that they secrete some product useful to the body. We are unable to define this substance further, but it may be a hormone or substance which, by acting on the pituitary gland, decreases gonadotropic activity and maintains functional equilibrium.

Klinefelter, Reifenstein and Albright (16), as a result of their findings in their syndrome, suggest that the seminiferous tubules produce a hormone which they call "X" hormone; they suggest that this "X" hormone is analogous to and probably very similar to estrin. These authors also point out that so much more testosterone is required to prevent development of castration cells than to affect seminal vesicles and prostate as to suggest that inhibition of formation of castration cells is not a physiological function of testosterone. In this connection mention should be made of the

finding of Huggins and Moulder (13, 14); these authors studied tumors of the Sertoli cells in dogs and they found such tumors rich in lipids and estrin: in one case they found an amount of estrogen as great as in ovaries containing large follicles at the height of estrus. Huggins and Moulder concluded that the Sertoli cells produce estrogen.

We regret that we have not measured the urinary estrin in our patients.

### SUMMARY AND CONCLUSIONS

1. Five cases are presented of a new syndrome, the chief clinical features of which are small testes and sterility in otherwise normal-appearing males.

2. Testicular biopsies showed complete lack of germinal epithelium, normal-appearing Leydig cells, and tubules with normal-appearing walls and containing normal-appearing Sertoli cells.

3. To explain the absence of the germinal epithelium it is hypothesized that the primary gonocyte failed to penetrate the embryonic testis.

4. Assays of urinary follicle-stimulating hormone were normal.

5. 17-ketosteroid excretions were somewhat low.

6. It is suggested that the Sertoli cells are the source of a hormone similar to estrogen, which regulates the follicle-stimulating hormone production by the pituitary.

### ACKNOWLEDGMENT

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# URINARY EXCRETION OF 17-KETOSTEROIDS IN VARIOUS CONDITIONS OF OLIGOPHRENIA CORRELATED WITH SOME AUTOPSY OBSERVATIONS

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URINARY 17-ketosteroid excretion as a measure of adrenal cortical activity plus male gonadal activity has attracted much attention in recent years, and standards for adults (6) and children (13) have been well established. As a method of diagnosing abnormal adrenal cortical activity in such conditions as hyperadrenocorticism (Cushing's disease), adrenal cortical tumors; hypoadrenocorticism (Addison's disease); male hypergonadism (Leydig cell tumors); and hypogonadism and panhypopituitarism the determination of 17-ketosteroids in the urine has considerable clinical importance. Hypogonadism in the male is reflected by low (female) values of 17-ketosteroid excretion, as seen in gynecomastia (9), cretinism, and mongolism (3, 13). Abnormalities in 17-ketosteroid excretion in certain psychoses, indicating a faulty adrenal cortical response to stress, have been observed by Hoagland and Pincus (8), and low values in diabetes have been reported by Miller and Mason (10).

The excretion of 17-ketosteroids in various types of mental deficiency has not yet been the subject of intensive research. Such a study seems of interest for several reasons. Although mental deficiency in its many degrees and variations does not represent a uniform morbid entity, observations indicate that it is very frequently associated with a general constitutional inferiority, malformations and anomalies of development occurring in many organs. While female morons are readily susceptible to impregnation, male morons usually appear sexually rather inactive and lower grade defectives are thought by many observers to be sterile. Of the forty-eight states of the Union, about thirty-one have some kind of sterilization law at present. Several northern European countries such as Denmark, Norway and Sweden have also accepted laws of this type. If the excretion of 17-ketosteroids in males were a true indication of gonadal activity, analyses would be helpful in selecting necessary cases and in determining the need or advisability of sterilization.

The material which is presented in the following pages is enhanced by a number of autopsies which were made on certain of the patients not long

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after their urinary 17-ketosteroids had been determined, or on patients of the same pathology, so that comparison between clinical observations and pathology has been possible.

### TECHNICAL PROCEDURE

**Collection of Urine Samples:** Twenty-four hour urine specimens were collected in bottles containing 5 or 10 ml. concentrated hydrochloric acid, depending on the volumes expected. They were kept as cold as conveniently possible during collection and then stored in the refrigerator for from a few hours to several days.

**Hydrolysis and Extraction:** Fifty ml. concentrated hydrochloric acid was added to exactly 500 or 550 ml. of the 24 hour specimens, which were then steam hydrolyzed and extracted with carbon tetrachloride according to the convenient method of Consolazio and Talbott (5).

Direct tests for completeness of the hydrolysis using androsterone benzoate and acetate were worthless, because these esters apparently were readily hydrolyzed by the alkali reagent even without preliminary acid hydrolysis. Nevertheless, equimolecular amounts of the benzoate on direct treatment with reagents gave an increase in color intensity due to the presence of the benzoate radical, whereas the benzoate added to a urine sample and subjected first to acid hydrolysis was recovered in amounts corresponding almost exactly to equimolecular amounts of non-esterified androsterone, indicating hydrolysis.

**Washings:** The carbon tetrachloride extracts were washed with 10 ml. saturated sodium bicarbonate, five 10 ml. amounts of 2 N sodium hydroxide, and three 25 ml. portions of distilled water.

**Drying and Decolorization:** To remove water and colored material the extracts were treated with 2 grams anhydrous potassium carbonate and 0.2 gram or less of Norit A for about 30 minutes, filtered through Whatman No. 43 paper, and washed with four 14 ml. portions carbon tetrachloride. It has been shown that under these conditions Norit A does not cause a significant loss of androgens from carbon tetrachloride solution as it does, for example, from ether solution.

**Evaporation and Solution:** With the addition of a few crystals of anhydrous potassium carbonate, the dried and decolorized extract was then evaporated in a water bath just to dryness, the carbon tetrachloride being recovered. Care was taken not to overheat the residue. The residue was dissolved in an exact volume (5, 10, or 15 ml.) of pure exactly 95 per cent alcohol, and filtered.

**Color Development:** Colors were developed by a semi-concentration modification of the Zimmermann reaction. Aliquots were measured into a 12 or 15 ml. capacity glass-stoppered centrifuge tube or graduate and

made up to exactly 2 ml. with 95 per cent alcohol. A blank of 2 ml. 95 per cent alcohol was also prepared. All tubes were placed in ice water in a beaker. When cold there was added quickly first 1 ml. 2 per cent meta-dinitrobenzene in absolute alcohol and then 1 ml. 15 per cent aqueous potassium hydroxide. The tubes were stoppered, inverted twice and removed at once to a water bath maintained at 25° C. and protected from daylight for 75 minutes. At the end of this time they were promptly placed in ice water again for three minutes after which there was added to each tube exactly 3 ml. of exactly 90 per cent pure ethyl alcohol. The tubes were inverted and shaken several times and solutions transferred to colorimeter tubes subsequently placed in ice water.

Readings: The colorimeter tube containing the blank was first carefully wiped dry and clean and placed in a Leitz photoelectric colorimeter (using a green glass filter transmitting at 520 mμ.) and the scale pointer set at 100. Then the sample tubes were similarly inserted and readings noted. 17-Ketosteroid content as androsterone was evaluated by comparison with a curve of readings of known solutions of androsterone.<sup>1</sup>

Following are data on duplicate determinations and recovery tests.

#### Duplicate Analyses of Urinary 17-ketosteroids as Androsterone

2.90 mg. vs. 2.88 mg.; 2.21 mg. vs. 2.23 mg.

4.90 mg. vs. 4.85 mg.; 3.85 mg. vs. 3.85 mg.

#### RECOVERY TESTS ON 500 OR 550 ML. URINE SAMPLES

Androgens as androsterone in sample	Added androsterone benzoate	Added androsterone	Total Found	Recovered as androsterone
mg.	mg.	mg.	mg.	mg.
4.13	—	0.50	4.65	0.52
2.26	0.50 equivalent to	0.37	2.60	0.34
2.26	2.50 equivalent to	1.84	4.05	1.79
1.25	—	2.50	3.75	2.50
1.25	—	2.50	3.75	2.50

In Table I are listed control cases of persons with normal intelligence. The urinary 17-ketosteroid excretion was found normal in all instances.<sup>2</sup> The thirty-eight year old male was a psychopathic personality with ab-

<sup>1</sup> The androsterone, androsterone acetate, and androsterone benzoate were kindly provided by Dr. Ernst Oppenheimer of the Ciba Pharmaceutical Products, Inc.

<sup>2</sup> Normal values of urinary androgen excretion, as mg. androsterone

Adult females: 5.1 to 14.2, average 9.0 mg./24 hrs. (6)

Adult males: 8.1 to 22.6, average 13.8 mg./24 hrs. (6)

Children under 8 years: less than 1 mg./24 hrs. (13)

Children 8 to 18 years: from less than 1 up to 9 mg. 24 hrs. (13)

Children 12 to 18 years: 1 to 9 mg./24 hrs. (13)



normal sex tendencies (transvestitism), and the second case was also a psychopathic personality. The third individual listed was an entirely normal control. The fourth control was a thirty-four year old woman in the fifth and sixth months of a pregnancy, respectively. She had had a mongoloid child two years previously. It is seen that her androgen excretion is normal in this pregnancy. The fifth patient, who was a woman of rather masculine features, with underdeveloped breasts and small pelvis, also had a normal value. The same is true of the last patient, who previously had had a hysterectomy and showed some signs of hirsutism.

TABLE I. TWENTY-FOUR HOUR URINARY ANDROGENS AS ANDROSTERONE:  
PERSONS WITH NORMAL INTELLIGENCE

Case No.	Sex	Age Yrs.	I.Q.	Vol. ml.	mg. per 24 hrs.	Remarks
1	M	38	normal	1362	17.3	Psychopathic personality. Transvestitism.
2	F	23	86	1001	8.3	Psychopathic personality.
3	F	25	normal	860	14.9	Laboratory worker.
4	F	34	normal	1600	9.1	Pregnant 5th month.
				1470	9.7	Pregnant 6th month.
5	F	42	normal	2625	6.7	Had mongoloid child at 32 yrs. Business woman. Nervous. Slight build. Question of physical underdevelopment.
6	F	45	normal	2935	11.8	Hystereectomy. Slight hirsutism.

The androgen values for twelve mentally deficient females are presented in Table II. Eleven of these were of familial or "hereditary" type. Nearly all of these patients had values well within the normal range (average 9.0 mg.), indicating normal adrenal cortical activity for this group. One of these girls, who was six months pregnant, had a value of 11.5 mg. per twenty-four hours, which, like the preceding instance, suggests an unchanged level during pregnancy. The two girls with psychopathic trend had normal amounts of urinary androgens. Patient No. 10, a rather asthenic, very blond girl of Norwegian descent, had a low value of 3.7 mg. The last patient, No. 18, with a low value of 2.3 mg. per twenty-four hours was an imbecile who became ill and died three months later.

The autopsy of case No. 18 revealed a white female, five feet five inches in height, with black, strong and abundant hair. Pubic hair was also abundant and there was hair on the upper lip and on arms and legs. The bony features were strong, the chin prominent. There was a striking asymmetry of the breasts, the right breast being rather firm but normal, the left small.

with a large areolar gland set on a ball-like glandular body without the usual subcutaneous fat tissue characteristic of the female breast. The right adrenal gland weighed 9.8 grams, the left 10.5 grams. Both were well developed; the cortex and medulla were distinctly outlined; the fasciculata contained a normal amount of lipoids. The ovaries were fairly large but undersized for an adult; both together weighed 19 grams; they appeared

TABLE II. TWENTY-FOUR HOUR URINARY ANDROGENS AS ANDROSTERONE:  
FAMILIAL-TYPE FEEBLEMINDED FEMALES

Record No.	Case No.	Age Yrs.	I.Q.	Vol. ml.	mg. per 24 hrs.	Remarks
5831	7	14	61	660	9.0	
5836	8	17	46	878	7.3	Slightly retarded physical development.
5850	9	18	59	710	13.4	Good physical development.
3618	10	19	62	1062	3.7	Asthenic. Very blond.
4942	11	19	40	1070	10.6	Homosexual tendencies.
5238	12	20	47	3080	9.0	Homosexual tendencies. Short; small face and shoulders; lower trunk obesitas, with striae.
4755	13	21	61	1215	5.4	
5588	14	22	67	845	6.9	Good physical development. Infection of tube and ovary. Uterus enlarged.
3959	15	23	57	1800	11.5	Pregnant about 6 months.
4255	16	23	55	1665	9.6	Treated for T.B. of spine.
5829	17	24	60	1075	13.0	Reddish blond. Grandmother mulatto.
3489	18	26	23	778	2.3	Tall. Masculine. Died three months later of bronchopneumonia and glomerulonephritis.
Average, excluding No. 18, is 9.0 mg./24 hrs.						

sclerotic and there was only one matured graafian follicle. The thyroid gland weighed 27 grams. The cause of death was bronchopneumonia and old glomerulonephritis.

In Table III twelve values are listed for eleven male patients with "familial type" of mental deficiency. As may be seen, the results for the patients above the age of fifteen (average 14.2 mg.) tend to fall within the normal range for adult males, with two exceptions. Case No. 28 had a low value of 5.3 mg. per 24 hrs., but a year later it rose to 11.7 mg., which is well within the normal range. The value of 26.9 mg. for case No. 24 of adult age is a little high, but may correspond to the large size and husky

development of this typical simple low-grade moron. Whether or not there is an increased variability in excretion for some of these patients requires further investigation. The findings for the younger boys are also within normal range for each age.

Several of the patients showed abnormal sex tendencies or conspicuous psychopathic characteristics, but these abnormal personality trends are not reflected in the 17-ketosteroid excretion.

TABLE III. TWENTY-FOUR HOUR URINARY ANDROGENS AS ANDROSTERONE:  
FAMILIAL-TYPE FEEBLEMINDED MALES

Record No.	Case No.	Age Yrs.	I.Q.	Vol. ml.	mg. per 24 hrs.	Remarks
5857	19	9	78	560	2.0	Borderline intelligence. Probable early nutritional deficiency.
5875	20	11	61	1190	4.7	
5920	21	12	66	815	4.5	
5384	22	14	53	1085	5.9	Homosexual tendencies.
5888	23	15	71	2280	14.4	Mature appearance. Psychopathic personality. Arsonist. Sex pervert.
5943	24	22	48	2278	26.9	Large build.
4695	25	25	69	3125	16.2	Psychopathic personality. Effeminate. Homosexual.
4355	26	25	50	1905	17.0	Effeminate. Homosexual.
4237	27	27	38	2145	12.9	Effeminate.
5879	28	28	43	1220	5.3	
		29		1730	11.7	
1728	29	38	56	1365	9.1	Small build. Good physical development. Part negro. Homosexual.
Average, No. 24 through No. 29, is 14.2 mg./24 hrs.						

In Table IV values for nine mongoloid patients are given, supplementing 23 cases previously reported (3). The female patients of from twelve to seventeen years of age had normal female values for this age. Case No. 35, twenty-nine years of age, also had a normal androgen excretion. Case No. 34, a female of twenty-one years had an unusually high value, above the normal female range and within the normal range for adult males. In our previous publication we reported two observations of this type. It is interesting, however, to note that the present patient had a value of 1.3 mg. only two months previously.

Of the male patients, one was a baby of four months whose 17-ketosteroid value of 0.4 mg. is a good value for a child of this age. One male mongoloid of thirty-four years had a value of 15.1 mg., which is higher than any value previously reported for mongoloid males, but is close to



mal. This was especially striking in the case of the male cretin, who had an almost hypertrophic escutcheon.

The first listed cretin (case No. 39) was a female dwarf, twenty-three years of age, with a body length of four feet. The patient did not reveal the usual features of cretinism, although there was evidence that the case should be classified as such. She was born by high forceps, 21 days overdue. The neck was badly twisted and there were multiple birth bruises on skull and body. The first month the child slept most of the time and did not cry. A few epileptic attacks were observed during the first year. The child had been treated with thyroid for some time early in life, but the dosage is not known. At the age of 14 years the child had a blood cholesterol of 400 mg. per cent and a basal metabolic rate of minus 11 per cent by height.

TABLE VI. TWENTY-FOUR HOUR URINARY ANDROGENS AS ANDROSTERONE:  
MYOTONIA DYSTROPHICA PATIENTS

Record No.	Case No.	Sex	Age Yrs.	I.Q.	Vol. ml.	mg. per 24 hrs.	Remarks
4570	43	M	31	68	1050	5.4	No. 43 and No. 44 are brothers.
3929	44	M	35	73	2165	8.0	
2702	45	M	34	51	775	6.6	No. 45, No. 46 and No. 56 are siblings.
2703	46	M	36	61	690	4.8	
2809	56	F	39	63	1255	2.8	Another sister was similarly afflicted.

After thyroid treatment for  $2\frac{1}{2}$  months the cholesterol dropped to a level of 157 mg. per cent and the metabolic rate rose to plus 9 per cent. One month after thyroid was withdrawn, the cholesterol was again up to a level of 351 mg. per cent and the metabolic rate down to minus 11 per cent. The patient had an unusual amount of hair on head, back and neck, but little pubic and axillary hair. She did not menstruate. Knee jerks were overactive; ankle jerks active. The skin was rough and showed slight ichthyosis.

The 17-ketosteroid finding for case No. 41, diagnosed as hypothyroidism, is also low.

Table VI records 17-ketosteroid determinations on a group of patients afflicted with myotonia dystrophica. Myotonia dystrophica is a familial muscular dystrophy with tonic reflexes, especially in the thenar group, associated with general weakness of other muscles. There is also progressive atrophy of certain muscles such as the temporal muscles and the muscles of the lower arm and leg. The muscular condition is associated with a wide-

spread endocrine disorder, the most striking feature of which is gonadal atrophy. Three autopsies made possible a thorough study of the pathology of the endocrine system in this disease. Consistent findings were testicular atrophy with hyalination of the seminiferous tubules; basophilism in the pituitary anterior lobe, increased basophilic infiltration of the posterior lobe with atrophy and colloid stasis in the Rathke cleft; colloid goiter; and persistent thymus with fatty infiltration. There was considerable general obesity.

The tests on the four male patients showed a definite decrease in 17-ketosteroid excretion. The values were all below the normal range, three falling within the lower range considered normal for females. The value of 4.8 mg. found in case No. 46 is even below the female range.

The first two male patients listed in this table were brothers who died a few months after the tests had been made, and were autopsied. The other two male patients and the female patient are siblings. These had a sister similarly afflicted who died and was autopsied.

Case No. 43 showed atrophy of the seminiferous tubules with hyalinosis of about 90 per cent of the tissue. There were few islands left where normal spermatogenesis was present. The Leydig cells were degenerated around the hyalinized tubules, but in those few islands which had normal spermatogenesis a number of Leydig cells were found. Case No. 44, who died six months after his brother and seven months after the 17-ketosteroid determination, was in relatively better condition at the time of the tests, but at the time of autopsy the gonadal atrophy had progressed much further than in case No. 43. The whole testicle was atrophied, with practically all tubules hyalinized and all Leydig cells replaced by connective tissue masses. In both patients the adrenals were unusually soft and fell apart in spite of most careful handling. The medulla was hyperemic with all vessels greatly enlarged; the medullary tissue was partly fibrotic. The adrenal cortex showed evidence of irregular degeneration, and lacked the rather uniform appearance seen in normal material. Areas of degeneration were irregularly dispersed among some of fairly normal appearance, but all lacked lipoid deposits. The zona fasciculata and zona glomerulosa were more involved than the zona reticularis. As a whole the cortex was narrow.

Most conspicuous was the pathology of the pituitary in these cases. "Crooke's changes" of the basophils, reduction of eosinophils (which were fairly well preserved only in case No. 43), proliferation of the basophils at the edge of the posterior lobe, large colloid cysts in the cleft and degeneration of the posterior lobe were observed in all. In the female patient mentioned in case No. 44 there were small chromophobic tumors in the anterior lobe. In all three cases the thyroid formed a huge colloid goiter which weighed in case No. 44, 75 grams and in case No. 43, 37 grams, the vesicles

being enlarged and filled with stagnant brittle colloid, the epithelial walls compressed and atrophic. The thymus showed predominantly fatty infiltration but some non-involuted preserved thymus tissue could be found in both patients. With regard to the other organs, it may be mentioned that the heart was enlarged and the heart muscle thoroughly degenerated, with hyalinization and fatty infiltration. The pancreas of case No. 44 showed necrosis. All organs showed a tremendous congestion; the periph-

TABLE VII. TWENTY-FOUR HOUR URINARY 17-KETOSTEROIDS:  
MISCELLANEOUS TYPES

Record No.	Case No.	Sex	Age Yrs.	I.Q.	Vol. ml.	mg. per 24 hrs.	Remarks
5939	47	F	18	51	1014	11.1	Psychosis. Obesity.
4592	48	F	24	27	1875	15.3	Lawrence-Moon-Biedl Syndrome.
1825	49	F	41	18	1475	4.0	Tuberous sclerosis. Dwarfism.
5201	50	M	9	40	2350	1.4	Congenital cerebral spastic infantile paralysis. Underdevelopment.
5848	51	M	13	57	1075	3.5	Post-infectious. (Whooping cough.)
5918	52	M	14	55	1355	6.3	Post-traumatic, natal.
5928	53	M	14	61	1060	6.3	Question of pituitary disorder and post-encephalitis. Sex problem. Transvestitism.
5923	54	M	15	36	1245	9.1	Post-traumatic, natal.
5563	55	M	20	33	1186	5.6	Constitutional inferiority. Spinal dysraphism.

eral vascular system at the time of death was collapsed and face and trunk were dark blue in color.

In Table VII, eight more values are listed for a miscellaneous group of feeble-minded patients. These determinations were made for various reasons. Case No. 47, a female of eighteen years eleven months, was thought to be hypothyroid and hypopituitary, contributing to a psychosis. The patient was extremely obese but under diet the obesity disappeared. The 17-ketosteroid excretion of 11.1 mg. per twenty-four hours was normal. Case No. 48, a very short female of twenty-four years with a Lawrence-Moon-Biedl syndrome, showed a rather high value of 15.3 mg. while the next patient, a female dwarf with tuberous sclerosis, showed the low value of 4.0 mg. per 24 hrs.

The following five male patients with evidence of brain damage had average values. Of considerable interest is patient No. 55 a male imbecile with

spina bifida and indications of spinal dysraphism. This condition is hereditary and if it should turn out in further tests that the low value of 5.6 mg. per 24 hrs. is representative and indicates gonadal hypoplasia in male cases, the determination of urinary 17-ketosteroid excretion may have considerable diagnostic value. Several autopsies have revealed gonadal hypoplasia in male patients with familial oligophrenia and spinal dysraphism. This male patient resembled clinically the type of the female patient No. 18, who had the extremely low value of 2.4 mg. per 24 hours.

### DISCUSSION

Determination of urinary 17-ketosteroid excretion in a group of patients with various conditions associated with mental retardation seems of considerable interest. Since urinary 17-ketosteroids are an index though not a measure of adrenal cortical activity and male gonadal activity, the determination of their amount promises some insight into the activity of these tissues in these patients. The present study encompasses several types of oligophrenic patients, some having conditions associated with profound endocrine pathology.

#### Non-Glandular Oligophrenic Types.

The normal values found for female familial morons support the clinical observation that, in these cases, adrenal cortical activity is normal and gonadal activity is probably normal also. Female familial morons are readily susceptible to impregnation and potentially fertile to a high degree. Male familial morons also had normal values and seem normal in so far as adrenal and gonadal physiology are concerned. Their mental slowness and lack of responsiveness makes them potentially less aggressive and leads frequently to homosexual rather than heterosexual activities, but these alterations are mentally conditioned and are not reflected in physiological or anatomical pathology (11). Cases of birth trauma with resulting spasticity or Little's disease also show normal 17-ketosteroid excretion.

#### Myotonia Dystrophica.

The study includes reports on myotonia dystrophica for the first time. Five patients were studied. All patients showed definitely low 17-ketosteroid excretion. Clinically the males had small, atrophic testicles, were of weak musculature and obese, but pubic and axillary hair was normal and the sexual characteristics of these patients remained unchanged. The endocrine pathology consisted in pituitary basophilism with Crooke's changes, complete atrophy of seminiferous tubules and atrophy of Leydig cells, thyroid colloid goiter, persistent fatty thymus, and general obesity. Myotonia dystrophica, therefore, shares certain patterns with better known endocrine



disorders such as Cushing's disease and gynecomastia. Indeed myotonia dystrophica is often associated with gynecomastia clinically. Klinefelter and co-workers have reported low 17-ketosteroids and high F.S.H. in gynecomastia. Albright recently made determinations of F.S.H. on two myotonia dystrophica patients and found high values. Patient *H.W.*, 33 years old, was positive for 96 and 192 mouse units, and patient *F.H.* also had a high F.S.H.<sup>3</sup> The high F.S.H. values found by Albright and the low 17-ketosteroids found in our studies indicate that myotonia dystrophica and gynecomastia follow similar endocrine patterns.

Cushing's disease is associated with high blood pressure while two of our patients with myotonia dystrophica had a very low blood pressure and died from peripheral vascular atony. Patients with Cushing's syndrome have an hypertrophic adrenal cortex according to Albright, who postulates an hyperexcretion of the "S-hormone" as the cause of the syndrome. A study of the adrenals in myotonia dystrophica did not reveal any hypertrophic changes in these organs. The adrenals were thin, soft and hypoplastic, and if there was any pathology of significance, it was on the degenerative and hypoplastic side. None of the cortical layers showed an increase in size. The low 17-ketosteroid values are amply explained by the testicular atrophy and by adrenal atrophy as demonstrated in the female patient, but whether the gonadal degeneration is primary, as is supposed in gynecomastia, or secondary to another more profound metabolic disorder as indicated by the widespread pathology, cannot be determined at present.

### Cretinism and Hypothyroidism.

The new determinations on cretins and hypothyroid patients fall in line with earlier determinations made by Talbot and others (13). The opportunity for performing autopsies on two patients of this type enables us to state that the low urinary 17-ketosteroid values in cretinism are the result of gonadal and adrenal infantilism which may be due to hypopituitarism as a result of absent thyroid function or vice versa. Some authors have the idea that the pituitary in cretinism shows a compensatory hypertrophy. This erroneous conception is based on the observation that the weight of the pituitary in cretinism is usually increased from fifty to one hundred per cent. Microscopic study of the pituitary reveals, however, that the gain in weight is due to colloid accumulation and fluid stagnation (edema), while the glandular tissue is compressed and hypoplastic. More details are given in another publication (2).

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<sup>3</sup> These data were placed at our disposal through the courtesy of Dr. Paul Yakovlev, Walter E. Fernald School, Waverley, Massachusetts.

### Mongolism.

The study of mongoloid patients shows that such children and females of adult age have average to high normal 17-ketosteroid excretion, while mongoloid males of adult age tend to have female values. These are, however, higher than those seen in cretinism, and the findings for several female mongoloid patients were unusually high. We expressed the opinion that in mongoloid males the relatively high values are probably due to adrenal cortical activity and not to normal male gonads, since these were found atrophic in all autopsies. Mongolism seems to be a congenital hypopituitarism and the growth disorder represents the opposite of acromegaly (A. Schüller (12) Clift (4), Benda (2)). Benda has collected evidence that the condition is associated with hypothyroidism, hypocorticoadrenalism and hypogonadism apparently due to absence of the tropic hormones of the pituitary, which fail to activate the target glands. Histological examinations of the adrenals in thirty-nine cases revealed that while the permanent cortex was small and hypoplastic in all cases, a considerable number of cases showed a large hyperplastic juxta-medullary zone. As has been discussed in another place (2), the hyperplastic juxta-medullary zone is apparently not a remnant of the fetal cortex, since all mongoloid patients who died in infancy showed complete involution of the fetal cortex and did not show remnants of the "central body." On the other hand, since it is generally assumed that the "reticularis" is a part of the permanent cortex and depends in its development upon the development of the fasciculata, it is not conceivable that an hypertrophic juxta-medullary zone is derived from a degenerated and hypoplastic "permanent cortex." One is, therefore, forced to postulate an independent ontogenetic and functional existence of this "x-zone," which is well described as "juxta-medullary zone." It may be remembered that this zone has attracted considerable interest since discovery of the adreno-genital syndrome. Grollman (7) called this tissue the "androgenic tissue" and considers it as a remnant of the fetal cortex. Persistence or hypertrophy in castrated animals has been observed by various authors. On the basis of physiological evidence, Albright *et al.* (1) postulate the existence of at least two independent and antagonistic adrenal cortical hormones, the S-hormone and the N-hormone, in normal adults. This idea seems well supported by histological observations which indicate strongly the independent existence and function of the permanent cortex and the juxta-medullary zone in the human adrenals throughout life. While the "permanent cortex" (glomerularis, fasciculata and reticularis) is serving certain metabolic functions concerned with fat and sugar metabolism and liver glycogen the "juxta-medullary" zone or inner reticularis seems to be geared into the pituitary-adrenal-gonadal

activity and changes, therefore, conspicuously in pathological conditions of the gonads.

### CONCLUSIONS

Determinations of urinary 17-ketosteroids in various conditions associated with mental deficiency seem to indicate that abnormal brain development as such has little influence upon endocrine factors related to urinary 17-ketosteroid excretion. Familial cases of mental deficiency as well as some cases due to various types of brain injury revealed normal values in so far as the twenty-four hour excretion was concerned. Whether differences would be found between night and morning excretions similar to those claimed for psychotic patients by Pincus has not yet been determined. The frequent sexual perversions observed in mentally deficient patients seem to be associated with mental factors rather than physiological variations. Deviations from the normal range of 17-ketosteroid excretion, when found in mental deficiency, indicate, therefore, some direct pathology of the glands concerned with 17-ketosteroid excretion, and not primarily subnormal brain activity, the endocrine function of which is still a matter of argument and speculation. Patients with marked constitutional inferiority, especially those with spinal dysraphism, seem to have an equal constitutional inferiority of their endocrine systems.

The opportunity of performing several autopsies on cases whose 17-ketosteroid excretions had been determined, and tests on patients who suffered from certain conditions where enough autopsies of similar pathology were available, throw some light on possible relations between abnormally low 17-ketosteroid excretion and pathology.

Observations on myotonia dystrophica show that the low excretion of 17-ketosteroids is consistent in both sexes. The pathology of two male and one female patient revealed gonadal atrophy. In the males there was marked hyalinization of the seminiferous tubules of the testicles, and atrophy of the Leydig cells though to a less extent. The ovary of the female patient (30 years of age) showed almost no primordial follicles in the process of maturation. There were, however, four larger and several smaller cysts side by side in the same ovary, which had failed to involute. The pituitary showed in all three cases a conspicuous shift toward basophilism with all basophilic cells enlarged, homogeneous and vacuolated, changes described by Crooke in Cushing's disease and known now under his name. In contrast to some investigators, the present writers are of the opinion that the "Crooke's changes" are not a manifestation of hyperactivity, but indicate a low stage of activity with inability of the cells to release the increased and stagnant hormonal stores. The hypertrophic changes of the Leydig cells as seen in gynecomastia are probably likewise a manifestation

of inactivity, since the active spermatogenic testicle has very few and inconspicuous Leydig cells. In addition, myotonia dystrophica patients develop a resting colloid goiter (weight, 75 grams in one case). The adrenals are small. The permanent cortex is narrow and resembles that of a child rather than of an adult. The "juxta-medullary" zone is also inconspicuous.

In mongolism, where gonadal atrophy or immaturity is complete, the 17-ketosteroids remain on a level normally reached before puberty. Some adult females have unexpectedly high values and some children have high values for their age. The permanent cortex of the adrenals in mongolism is narrow and infantile, but in a study of 38 cases a surprisingly large number showed a conspicuous "juxta-medullary" zone.

In cretinism, where the 17-ketosteroid values are always low, the adrenal cortex is narrow and the innermost zone of the reticularis inconspicuous. The anatomical observations, therefore, seem to support the idea that the adrenal cortex produces two entirely independent hormonal factors. We dare say that the 17-ketosteroid production is related to a "juxta-medullary" zone, the inner reticularis which exists in man throughout life and is not merely the oldest cell stage of the permanent cortex.

Our observations provide evidence that in patients with low 17-ketosteroid excretion, the innermost zone of the reticularis may be expected to be found narrow and inconspicuous, while the outer layers may be of normal size. In other conditions the outer layers remain narrow or hypoplastic, while the inner layer of the cortex appears large. In these latter cases urinary 17-ketosteroids may be normal or increased. Increase in the "juxta-medullary zone" of the adrenals is not necessarily associated with gonadal hypertrophy. On the contrary, in cases where the gonads are unable to respond on account of immaturity or other physiological factors, the adrenals are likely to respond with such enlargement. In general, "hypertrophic" glandular changes are frequently not the manifestation of secretory hyperactivity, but of an inability to release secretory stores.

#### SUMMARY

- (1) Twenty-four hour urinary excretion of 17-ketosteroids was studied in several conditions of mental deficiency with and without endocrine disorders.
- (2) Urinary 17-ketosteroid excretion was found normal for familial morons of both sexes, consistent with normal adrenocortical and gonadal activity.
- (3) Myotonia dystrophica was found to be associated with a marked decrease in urinary 17-ketosteroids, consistent with the pathology.
- (4) Three cretins and an hypothyroid patient had low 17-ketosteroid

excretion, in line with previous reports in the literature of low androgens in hypothyroidism.

- (5) Findings for nine mongoloid patients were in agreement with those for twenty-three cases reported earlier. Mongoloid children and female mongoloids had low average or high 17-ketosteroid excretion. Mongoloid males of adult age have a 17-ketosteroid excretion in the female range, consistent with their known sexual immaturity.
- (6) Autopsy findings have been considered in relation to the 17-ketosteroid excretion and the significance of these observations has been discussed.

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# ESTRONE CLEARANCE TEST IN INFECTIOUS HEPATITIS\*

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**Z**ONDEK (11) showed in 1934 that the living organism inactivates estrone rapidly. Of an injected dose of estrone, only 5 per cent can be recovered from the body three hours after the injection. Liver in vitro inactivates estrone to an extent of 95 per cent, and spleen to an extent of 20 per cent, whereas the other organs in vitro have no effect on estrone. Estrone is also inactivated by poikilothermal animals, by hyacinth root (10), potato juice, red beet, bran, and certain bacteria (12,13). Cell-free extracts of liver inactivate estrone, but lose this activity on heating to 70° (11). It may be concluded, therefore, that the inactivation of estrone is enzymatic in nature. The enzyme which is responsible for this inactivation has been called "estrinase." Zondek and collaborators (13, 14) proved that estrinase from plants (potato juice) belongs to the phenol-oxidase group, being distinct from laccase, but related to tyrosinase.

The above experiments on the site and nature of estrone inactivation have been confirmed by many other authors. It has been shown by a wide range of methods that the liver is concerned in estrone inactivation and that impairment of its function interferes with the normal estrone metabolism of animals and man.

In 1938 Golden and Sevringhaus (3) showed that cyclic sexual function in the rat is completely suppressed if the ovaries of the animal are transplanted to mesenterial tissue at a site which ensures the passage of all the ovarian secretion through the liver.

In 1939 Talbot (9) pointed out that poisoning of the liver with CCL<sub>4</sub> results in increase of the endogenous estrone level. In 1940 Pincus and Martin (7), using the same technique, showed that response to administered estrone is heightened by liver damage. In the same year Heller (4) reported that rat liver slices are able to effect complete inactivation of estrone and estradiol in vitro. Biskind (1), in 1941, showed that pellets of estradiol and estradiol benzoate when implanted into the spleen of female castrated rats are ineffective unless blood which leaves the spleen is diverted into the general circulation. Israel, Meranze and Johnston (1937) (5) showed by heart-lung and liver-lung perfusions, and Schiller (8) (1945)

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showed by perfusions of heart, kidney and liver that the liver is largely responsible for estrogen inactivation, but that some inactivation is also effected by the kidney.

In 1940, Glass, Edmondson and Soll (2) reported that cirrhotic human liver fails to inactivate estrogens. Urine of advanced cases of liver cirrhosis was found to have a high free estrogen content. An etiological connection between this finding and testes atrophy and gynecomastia was envisioned. Morrione (6), in 1944, confirmed this by his finding that atrophy of the testes is the most constant feature of Laënnec's cirrhosis.

During the period of 32 months from October 1943 to July 1946, epidemics of infectious hepatitis, whose peaks fell chiefly between the months of October and December, occurred in Palestine. During this period twenty-nine cases of pregnancy complicated by infectious hepatitis were admitted to our department. When associated with pregnancy infectious hepatitis showed a very high mortality rate. In 17 per cent of the patients the disease ran a fatal course and death set in under hepatic coma. In these cases the post mortem examinations disclosed liver atrophy with almost complete destruction of the liver tissue.

Since production of sex hormones in pregnancy is known to be high, (Aschheim and Zondek) the fate of the endogenous and parenterally administered estrogens in pregnancy complicated by infectious hepatitis (resulting in acute liver damage) is of particular interest.

Experiments were undertaken with a view to ascertaining a) whether pregnant women with infectious hepatitis show an abnormally high level of endogenous estrogen in their blood and urine, and b) if the acute liver damage affects the rate of the excretion of parenterally administered estrone.

Information on these points was obtained with the help of the estrone clearance test described below.

#### Technique of estrone clearance test.

Urine and blood from 25 patients was examined for estrogen according to the method of Allen and Doisy. Five animals were used in each determination. The test dose was given subcutaneously in six injections distributed over a period of 48 hours. Vaginal smears were taken at 12-hour intervals between the 24th and 96th hour after the experiment was begun. The M.U. was defined as that quantity of urine and blood which produces full cornification between the 72nd and 96th hour of the experiment (1 M.U. is equivalent in our local strain of mice to 2 I.U.). Urines collected during an interval of 4-5 days preceding the injection of estrone were pooled for the estrogen assay. The results were calculated to a basis of 24 hours.<sup>1</sup> At the same time the serum estrogen titer was determined.

<sup>1</sup> At the beginning urines were investigated for estrogen content daily. As the daily

After the titers of endogenous estrogen (i.e., the estrogen titer before estrone injection) in the urine and serum had been determined, the estrone clearance test could be started. The patient was injected intragluteally with 25 mg. of estrone in oil, an equivalent of 250,000 I.U. (125,000 M.U. in our local strain of mice). Four hours after estrone injection, blood was drawn and examined for its estrogenic content. Urine excreted during the 3-day period following the injection was investigated as to estrogen content daily. The estrone clearance was estimated in terms of the excess of the estrone excretion during this period over the normal excretion value. The result is expressed as a percentage of the injected amount of the hormone.

### Cases investigated.

The estrone clearance test was performed in: a) cases with no apparent liver damage (control group) (Table B, cases No. 14-24), b) cases of infectious hepatitis in pregnancy showing moderately acute liver damage (Table A, cases No. 1-7), c) cases of infectious hepatitis in pregnancy showing extremely acute liver damage (Table A, cases No. 8-11), d) cases of chronic liver damage (Table A, cases No. 12, 12a, 13).

### Description of cases and findings.

a) This group of cases, served as the controls. (See Table B, cases No. 14-24.) The group includes a case of normal pregnancy, 4 cases of toxemic pregnancy, 3 cases of riboflavin deficiency, one case of acromegaly and one case of pregnancy complicated by Morbus Gaucher. Liver function tests (Cephalin test, Takata-Ara, V. den Bergh, urinalysis for urobilin and urobilinogen) failed to disclose any evidence of hepatic damage in any of these patients.

In Table B the endogenous estrone levels and clearance tests of the control group are presented. It is evident that administered estrone disappeared very quickly from the organism. Four hours after injection of estrone the pre-injection level of the blood was already restored.<sup>2</sup> Most of the excretion of estrone in the urine occurred during the first 24 hours. Some estrone was also excreted in a few cases on the second day, but always in smaller amount. The total urinary recovery of the injected estrone ranged between 0.5-4.5 per cent. These results suggest that failure to

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excretion of endogenous estrone was found constant, a simplification was introduced in the later work. Urine excreted during 4-5 successive days was pooled and the estrogen content was determined in the total sample.

<sup>2</sup> The normal endogenous estrogen values in blood during pregnancy are as follows: 50-100  $\mu\text{U/L}$  in the 1st and 2nd month, 100-800  $\mu\text{U/L}$  in the 3rd to 7th month and 800-1500  $\mu\text{U/L}$  in the 7th to 10th month. The normal estrogen values in urine during pregnancy are as follows: 300-600  $\mu\text{U/L}$  in the 1st to 2nd month, 5000-7000  $\mu\text{U/L}$  in the 3rd to 7th month and 6000-20,000  $\mu\text{U/L}$  in the 7th to 10th month.



TABLE A. ENDOGENOUS ESTROGEN ESTIMATION AND ESTRONE CLEARANCE TESTS IN  
INFECTIOUS HEPATITIS AND CIRRHOSIS

Description of patient	Estrogen values in urine and serum						Total recovery of estrone in urine	Outcome of cases	Chemical blood findings				
	Endogenous values		Values after injection of 25 mg estrone						21-36 mg%	Cholesterol Total	Cholesterol Ester	Cholesterol Free	Sugar
	24 hr. urine	Serum	4 hr. serum	1st day urine	2nd day urine	3rd day urine							
1. Infectious hepatitis in Grav. M. IV	500 $\mu$ U			5000 $\mu$ U	750 $\mu$ U	750 $\mu$ U	ca. 4%	recovered	128 mg%	52 mg%	76 mg%	80-120 mg%	
2. Inf. hepatitis, 2 weeks after delivery	<200 $\mu$ U			1000 $\mu$ U	400 $\mu$ U	200 $\mu$ U	ca. 1%	recovered	203 mg%	87 mg%	116 mg%	85 mg%	
3. Infectious hepatitis in Grav. M. VI	5000 $\mu$ U	1000 $\mu$ U/L	1000 $\mu$ U/L	10000 $\mu$ U	5000 $\mu$ U	5000 $\mu$ U	ca. 4%	recovered	152 mg%	74 mg%	78 mg%	64 mg%	
4. Inf. hepatitis, 6 days after interruption of Grav. M. VII	500 $\mu$ U	<250 $\mu$ U/L	<333 $\mu$ U/L	1000 $\mu$ U	1000 $\mu$ U	<500 $\mu$ U	ca. 1%	recovered	160 mg%	96 mg%	64 mg%	67 mg%	
5. Inf. hepatitis after interruption of Grav. M. VI		500 $\mu$ U/L	500 $\mu$ U/L					Patient died from uterine rupture	145 mg%	59 mg%	86 mg%	72 mg%	
6. Inf. hepatitis, 10 days after delivery	<200 $\mu$ U	<200 $\mu$ U/L	<200 $\mu$ U/L	1400 $\mu$ U	2000 $\mu$ U		ca. 3%	recovered	152 mg%	86 mg%	66 mg%	93 mg%	
7. Grav. M. IX. Inf. hepatitis one year ago; Persistent liver damage	7500 $\mu$ U	1000 $\mu$ U/L	1000 $\mu$ U/L	10000 $\mu$ U	10000 $\mu$ U	7500 $\mu$ U	ca. 4%	recovered	212 mg%	142 mg%	70 mg%	102 mg%	
8. Infectious hepatitis Grav. M. III	<200 $\mu$ U	<500 $\mu$ U/L	<500 $\mu$ U/L	7700 $\mu$ U	<1000 $\mu$ U		ca. 6%	recovered	111 mg%	53 mg%	58 mg%	70 mg%	
9. Infectious hepatitis in Grav. M. VI	5000 $\mu$ U			15000 $\mu$ U	10000 $\mu$ U	10000 $\mu$ U	ca. 16%	recovered	158 mg%	47 mg%	111 mg%	76 mg%	
10. Infectious hepatitis post partum			8000 $\mu$ U/L	12500 $\mu$ U	3000 $\mu$ U		ca. 12.5%	Patient died	247 mg%	105 mg%	142 mg%	72 mg%	
11. Infectious hepatitis in Grav. M. VI		1000 $\mu$ U/L	4500 $\mu$ U/L					Patient died	162 mg%	70 mg%	92 mg%	70 mg%	
12. Chronic hepato-splenomegaly	<200 $\mu$ U			11000 $\mu$ U	500 $\mu$ U	<500 $\mu$ U	ca. 9%	Patient died					
12a. Chronic hepato-splenomegaly	<200 $\mu$ U			15000 $\mu$ U	1600 $\mu$ U	<500 $\mu$ U	ca. 13.5%	Patient died					
13. Cirrhosis hepatitis	<200 $\mu$ U			7000 $\mu$ U	500 $\mu$ U		ca. 6%	recovered					

\* Normal values

\* Normal values.

restore the normal endogenous estrogen blood level within a period of four hours after hormone injection, with urinary recoveries in excess of 4.5 per cent, reflect an impaired estrone inactivation mechanism.

b) In this group are included seven cases of pregnancy complicated by infectious hepatitis with a clinical course similar to that observed in the absence of pregnancy. The infectious hepatitis appeared in various months of pregnancy or after delivery. Although the icterus index was greatly elevated in the majority of cases, the general condition remained satisfactory; fetor hepaticus, tachycardia, and reduced liver were not found. Nevertheless, liver function tests indicated the presence of serious liver damage. The ratio of cholesterol ester to free cholesterol in the blood and the sugar metabolism were abnormal. The mechanism of urea, on the other hand, was undamaged (normal blood urea levels). The mechanism of estrone inactivation was intact, in all these cases. The blood level of endogenous estrogen and the urinary excretion of endogenous estrone remained normal. In the estrone clearance test the pre-injection estrone titers were found restored in the blood 4 hours after the injection. The total recovery of estrone in the urine never exceeded 4 per cent, the normal value of the control group. The clinical course was only slightly more severe than is generally the rule in icterus infectiosus not complicated by pregnancy. All the patients, except one, recovered. The single fatality (case No. 5) was due to uterine rupture during delivery. No evidence of atrophy of the liver was found in this case at autopsy.

c) This group included four cases (Table A, cases No. 8-11) of infectious hepatitis complicated by pregnancy with signs of acute liver damage. The clinical course reflected pronounced functional deficiency on the part of the liver; the prognosis was extremely serious. The severity of these cases is shown both by the grave clinical development and by the liver function tests. The patients were admitted with infectious hepatitis (icteric phase) in different months of pregnancy (see Table A). The clinical symptoms were similar in all these cases: intense icterus, nausea, frequent vomiting, apathy, prostration, subcomatous condition, tachycardia<sup>3</sup> of 110-120 per minute, no fever, and no pruritus. At the time of admission the liver was found reduced in size in every case. Patients presented also fetor hepaticus, a sign of severe liver damage. The clinical signs of acute failure of liver function were further confirmed by laboratory findings, among which particular importance relates to the value of blood urea level. Ability to synthesize urea persists even after hepatic tissue destruction is far advanced. The progressive fall of urea blood level observed in the cases (Nos. 8-11 of group c) must be interpreted, therefore, as an indication of a condition of very extensive hepatic damage.

<sup>3</sup> An especially ominous symptom in our cases of infectious hepatitis.

TABLE B. ENDOGENOUS ESTROGEN ESTIMATIONS AND ESTRONE CLEARANCE TESTS IN CONTROL CASES

No.	Description of patient	Estrogen values in urine and serum						Total recovery of estrone in urine
		Endogenous values		Values after injection of 25 mg. estrone				
		24 hr. urine	Serum	4 hr. Serum	1st day urine	2nd day urine	3rd day urine	
14.	Pregnancy M. VIII	5000 MU		500 MU/L	10000 MU	5000 MU	5000 MU	ca. 4%
15.	Toxemia in pregnancy M. VIII	6000 MU	1500 MU/L	1500 MU/L	12000 MU	6000 MU	6000 MU	ca. 4.5%
16.	Toxemia in pregnancy M. VIII	<1000 MU	<500 MU/L	<500 MU/L	2000 MU	<2000 MU		ca. 2%
17.	Toxemia in pregnancy M. IX	4000 MU	<500 MU/L	<500 MU/L	8500 MU	4500 MU	4500 MU	ca. 4%
18.	Toxemia in pregnancy M. VIII	7500 MU	1000 MU/L	1000 MU/L	12000 MU	9000 MU	7000 MU	ca. 4.5%
19.	Metrorrhagia Galeatorrhea Hyperplastic Mucosa uteri	<200 MU	<500 MU/L	<500 MU/L	2000 MU	1000 MU	450 MU	ca. 3%
20.	Acromegaly	<333 MU			1000 MU	1000 MU	500 MU	ca. 2%
21.	Vitamin A & B deficiency	<200 MU	<200 MU/L	<200 MU/L	<500 MU	<500 MU	<500 MU	<1%
22.	Riboflavin deficiency	<120 MU	<250 MU/L	<250 MU/L	420 MU	250 MU		ca. 0.5%
23.	Riboflavin deficiency	<200 MU	<500 MU/L	<333 MU/L	660 MU	<500 MU		ca. 0.5%
24.	Morbus Gaucher in grav. M. VIII	1300 MU	500 MU/L	500 MU/L	1800 MU	1600 MU	<1400 MU	ca. 0.5%

Case No. 8. Mrs. M.M., 35 years old, was admitted for infectious hepatitis in the 3rd month of pregnancy. The clinical examination at admission pointed to the presence of infectious jaundice in a particularly severe form: pronounced apathy, excessive vomiting, tachycardia, and distinctly reduced liver (as demonstrated by percussion of the liver area). The laboratory findings revealed serious liver damage: cephalin test ++, Takata-Ara ++, Muelengracht 300, V. den Bergh direct +++. The general condition of the patient deteriorated rapidly, the liver diminished progressively in size and fetor hepaticus set in. The laboratory tests revealed rapid drop of the blood urea level from 21 to 7.5 mg. per cent, a hypoglycemia of 70 mg. per cent which was not influenced by massive sugar therapy, and a depression of the total cholesterol level to 111 mg. per cent,<sup>4</sup> and of cholesterol ester fraction to 53 mg. per cent, free cholesterol 58 mg. per

<sup>4</sup> In normal pregnancy we found the following plasma cholesterol values: Total

cent, free/ester ratio amounting to ca. 1. In spite of these findings, reflecting very pronounced liver damage, the endogenous estrone levels of the blood and urine remained normal (see Table A). The estrone clearance test rated 6 per cent at this stage. Since the normal recovery rate in healthy individuals is 0.5 per cent–4.5 per cent, this is consistent with moderate deficiency in the estrone inactivation mechanism. During the two following days the patient was in a comatous condition, and her general symptoms (hepatic fetor, tachycardia and vomiting) were much aggravated. Generous intravenous administration of sugar, blood and plasma seemed, however, to have good effect. The condition of the patient began to improve on the eighth day; the normal area of liver dullness became again discernible. The patient recovered after an illness of severe clinical course lasting 4 weeks.

*Case No. 9. Mrs. N.S.*, 20 years old, was admitted in the seventh month of her second pregnancy. Patient's history did not reveal serious previous disease. A week before admission loss of appetite, frequent vomiting and pains in the upper right of the abdomen were first experienced.

Patient appeared undernourished and apathetic. On percussion the dullness of the hepatic area was distinctly diminished and the spleen was felt soft and enlarged. The scleras and skin were deeply icteric. V. den Bergh ++, bilirubin in urine ++, blood urea 21 mg. per cent, cephalin test +, Takata-Ara +, total blood protein 5 per cent. During the next few days, the general condition became distinctly aggravated with pronounced apathy, extreme asthenia, and fetor hepaticus. Six days after admission coma set in and the hepatic dullness area disappeared completely. The laboratory findings showed extreme liver damage demonstrated by a rapid fall of the blood urea to 4.8 mg. per cent. Cholesterol total 158 mg. per cent, cholesterol ester 47 mg. per cent, free cholesterol 111 mg. per cent; free/ester ratio = 2.3, blood sugar 76 mg. per cent. In spite of these findings, the urinary endogenous estrogen level remained normal.

The estrone clearance test revealed pronounced inability on the part of the liver to inactivate estrone. The recovery rate was 16 per cent—an extremely high value. Generous sugar, blood and plasma therapy proved successful, and the patient eventually recovered.

*Case No. 10. Mrs. S.G.*, 35 years old, was admitted in the ninth month of her fifth pregnancy. For two weeks before her admission the patient complained of abundant vomiting and virtual inability to retain food. This had contributed to a state of distinct undernourishment. The patient felt sick and drowsy and complained of pains in the right upper abdomen. Jaundice had developed shortly before admission. Delivery pains appeared at the same time. A rapid and uneventful delivery uncomplicated by bleeding was achieved but was soon followed by signs of very serious liver damage (coma, fetor hepaticus, tachycardia and progressive and rapid reduction of the size of the liver). Urea 6 mg. per cent, total cholesterol 247 mg. per cent, cholesterol ester 105 mg. per cent, free cholesterol 142 mg. per cent, free/ester ratio = 1.35, glucose 72 mg. per cent, cephalin test + + + + +, Takata-Ara ++, Meulengracht 300. The estrone clearance test revealed a definitely deficient estrone inactivation, the recovery rate in urine being 12.5 per cent. Four hours after the estrone injection, the blood estrone level was still high (8000 mU/L in comparison to a normal maximal estrone level 1–2 days postpartum of 1000 mU/L). Therapy with large amounts of blood proteins and sugar, supported by methionine and vitamins, failed. At autopsy typical signs of acute atrophy of the liver were found.

cholesterol 205 mg. per cent, cholesterol free 65 mg. per cent, cholesterol ester 140 mg. per cent, free/ester ratio 0.15

*Case No. 11.* Mrs. S.Z., 27 years old was admitted in the middle of the seventh month of pregnancy. Nine days prior to admission, chilly sensations and fever had been experienced. On the third day of these complaints, the patient suffered from pains in the hepatic area, loss of appetite and frequent vomiting. The urine became darker. At admission marked jaundice of scleras and the skin was evident. The patient was very sick, appeared to be undernourished, and presented a distinctly reduced liver.

The blood urea level was 14 mg. per cent, cephalin and Takata-Ara tests strongly positive, icterus index 100, and v. den Bergh direct + + +. Profound apathy, extreme weakness and frequent vomiting. Pector hepatiens and a subcomatous condition set in. The urea blood level dropped progressively reaching 5.1 mg. per cent on the fifth day after admission. Cholesterol total 162 mg. per cent, cholesterol ester 70 mg. per cent, cholesterol free 92 mg. per cent, free/ester ratio 1.3, glucose 70 mg. per cent, despite massive intravenous sugar therapy. Following injection of 125,000 m.u. of estrone a high estrone level was still found in the blood after 4 hours, (4500 m.u. as compared to 1000 mU/L prior to the injection). The patient delivered on the next day prematurely and succumbed shortly afterwards in hepatic coma. The autopsy showed typical signs of acute hepatic atrophy with reduced liver weighing 850 Gm. and widespread degeneration of liver parenchyma.

d) This group includes two cases in which the liver damage appeared after a prolonged period of hepatic disease. In case No. 13 (Table A) the liver damage was due to a liver cirrhosis of 7 years' standing due to chronic alcoholic intoxication of the Laënnec type, characterized clinically by a poor general condition, emaciation and enlarged liver and spleen.

In the case 12, 12a cirrhosis of the liver was of unknown etiology and manifested clinically in chronic hepato-splenomegaly of several years duration, recurrent epistaxis, subicteric tinge of the scleras, anorexia and cachexia. The general symptoms aggravated progressively; one month after the admission hepatic coma developed and death followed. Both patients of the group presented deficiency of the estrone inactivation mechanism. The endogenous estrone level of the urine was normal (less than 200 mU/L), but the estrone clearance after injection of 125,000 m.u. was distinctly elevated; in case No. 13, 6 per cent of the injected estrone was excreted, and in the more severe case (case No. 12) 9-13.5 per cent was excreted. In the last case the estrone inactivation test was performed twice, the first time immediately after the admission and again just before the patient died a month later. The findings in estrone clearance reflected the deterioration of the general condition, the recovery value rising from 9 per cent in the first test to 13.5 per cent in the second clearance test.

#### DISCUSSION

Since the liver is the principal site of estrogen inactivation it was expected that in our cases of acute and chronic liver damage abnormally high blood and urine levels of endogenous estrogen as well as deficiency in ability to inactivate administered estrogen would be presented. Investiga-

tion of this problem seemed to us of particular interest since in the past experiments on this subject were mostly performed on animals.

Our cases of infective hepatitis in pregnancy were divided into two main groups. Patients in whom a severe illness was associated with failure of the liver to synthesize urea as well as failure of other liver functions were included in group c). On the other hand, patients with functional derangement of the liver (positive cephalin and Takata-Ara tests, drop of total cholesterol and cholesterol ester blood values, hypoglycemia, etc.) but without abnormality in urea synthesis, with generally a less serious clinical course, were classified as group b). Normal levels of endogenous estrogens in the blood and urine were observed not only in moderately severe cases but also in the cases which developed hepatic coma and terminated in death. The fact that endogenous normal levels were shown also by patients suffering from very serious liver damage, even after hepatic coma intervened, suggests that acutely diseased liver in an advanced stage of atrophy is still able to inactivate the large amounts of endogenous estrogens produced by the body during pregnancy, or, alternatively, that some other organ (spleen?) takes over this function. The inactivation of the parenterally administered estrone, in contrast to that of endogenous estrone, depended largely upon the extent of liver damage. In the moderately severe cases of infectious hepatitis the liver of the patients effectively inactivated 125,000 m.u. of injected estrone, within an interval of four hours. The urinary estrone recovery after injection was quite normal, i.e., never greater than 4.5 per cent. It may be concluded, therefore, that a moderately severe liver damage involving both failure of cholesterol esterification and impairment of the metabolism of glycogen and protein (hypoglycemia, hypoproteinemia, cephalin and Takata-Ara tests positive) does not necessarily entail any significant failure in the estrone inactivation mechanism.

On the other hand, patients who suffered from serious failure of liver function, characterized by failure of urea synthesis, a function which is generally the last to be extinguished when liver fails, presented a deficiency in estrone inactivation demonstrated both in a high urinary estrone recovery (estrone clearance of more than 4.5 per cent) and persistence of a high blood estrogen level four hours after hormone injection. Urea synthesis is thus the only important liver function which varies in a manner comparable to estrone inactivation function during infectious hepatitis complicated by pregnancy.

In one case of acute liver atrophy (case No. 8) with very low blood urea level (7.5 per cent) and a very serious clinical course, the estrone clearance rate was only slightly higher than the normal. Despite the grave prognosis, this patient recovered after a prolonged and very severe illness. This find-

ing raises the hope that the estrone clearance test may be of more significant prognostic value in acute liver disease than is the loss of urea production.

The ability of liver in hepatic chronic diseases to inactivate estrone was tested in 2 cases of cirrhosis of very long duration. Both these patients showed deficiency in the mechanism of estrone inactivation. The level of endogenous estrone in the urine was in these cases, as in the acute liver diseases cases, entirely normal (less than 200 mU/L). However, the estrone clearance test revealed in both cases inability on the part of the liver to inactivate injected estrone at a normal rate: the recovery of injected estrone in the urine was 6 per cent in case No. 13 and 9–13.5 per cent in the more severe case (case No. 12, 12a) which had a fatal outcome. In this last case, moreover, the estrone clearance test was performed twice (once before and again after the onset of hepatic coma). Increased excretion of estrone was found to accompany the aggravation of the clinical condition of the patient. This result suggests the possibility that in chronic liver diseases, repeated estrone clearance tests may prove useful as an indicator of the severity of the clinical course and thus help to establish the prognosis.

It may be concluded from the findings that estrone clearance measurement is a valuable adjuvant test of the degree of liver damage. Inability to inactivate estrone in a patient with liver disease precludes an ominous outcome.

#### SUMMARY

1) We have investigated the capacity of 24 patients with liver damage to inactivate estrogenic hormone. These patients included 13 in whom the liver damage was caused by infectious hepatitis occurring during pregnancy and several in whom the damage was caused by cirrhosis.

2) Extensive damage of the liver by acute (infectious hepatitis in pregnancy) or chronic (cirrhosis) liver diseases was not associated with an increased endogenous estrone level in the blood and urine.

3) Injected estrone disappeared normally from the organism very rapidly. Four hours after estrone injection, the pre-injection estrone level of the blood was already restored. In urine the main excretion of estrone occurred in the first 24 hours.

4) The amount of estrogens excreted in the urine by normal individuals ranged from 0.5 per cent to 4.5 per cent of the injected hormone amount.

5) Estrone clearance in patients with infectious hepatitis complicated by pregnancy is increased only in very advanced stages of the disease (comatous or pre-comatous condition) characterized by loss of ability to synthesize urea. The ability to inactivate estrone is among the last liver functions to be extinguished in progressive acute damage of the liver. Ability to inactivate estrone even outlasted the function of urea production.

6) The estrone clearance rate is abnormally high in both moderate and severe cases of cirrhosis of the liver. In a very severe case the clearance rate increased parallel with the aggravation of the clinical condition of the patient.

7) It is probable that the estrone clearance test can be a useful aid in the establishment of the prognosis of patients who suffer from acute or chronic liver disease. Persistence of elevated estrogen levels in the blood and urine after an injection of the hormone is an ominous sign in acute and chronic liver diseases.

#### ACKNOWLEDGMENT

We are indebted to Dr. Y. M. Broberg for his devoted clinical assistance.

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# ANNOUNCEMENT OF THE 1948 MEETING OF THE ASSOCIA- TION FOR THE STUDY OF INTERNAL SECRETIONS

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The Thirtieth Annual Meeting of the Association for the Study of Internal Secretions will be held in the Palmer House, Chicago, Illinois, June 18 and 19, 1948.

The scientific sessions will be held in the Red Lacquer Room and registration will be on the fourth floor just outside the Red Lacquer Room. The Annual Dinner will be held in the same room on Friday, June 18th at 7 p. m. and will be preceded by a cocktail party, the location of which will be announced later. The Council will meet at 2 p. m. Thursday, June 17th.

All members of the Association who plan to attend the Thirtieth Meeting are urged to make their reservations at once with the Palmer House, stating the time of arrival and how long they plan to remain in Chicago.

## Abstracts of

# CURRENT ENDOCRINE LITERATURE

*Editor*; D. A. MCGINTY. *Collaborators*: A. R. ABARBANEL, F. N. ANDREWS, B. L. BAKER, F. A. DE LA BALZE, ISRAEL BRAM, R. A. CLEGHORN, RUCKER CLEVELAND, C. D. DAVIS, ANNA FORBES, M. B. GORDON, H. S. GUTERMAN, M. M. HOFFMAN, R. G. HOSKINS, C. D. KOCHAKIAN, H. S. KUPPERMAN, H. L. MASON, JANET W. MCARTHUR, THOMAS H. MCGAVACK, A. E. MEYER, K. E. PASCHKIS, A. B. PINTO, J. R. REFORZOMENBRIVES, E. C. REIFENSTEIN, JR., G. G. RUDOLPH, L. T. SAMUELS.

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## PITUITARY

MEITES, J., AND C. W. TURNER. Effect of thiouracil and estrogen on lactogenic hormone and weight of pituitaries of rats. *Proc. Soc. Exper. Biol. and Med.* 64 (4): 488-492 (1947).

The administration of 0.1% thiouracil in the feed for 24 days to young female rats reduced the lactogenic hormone content of the pituitary below that in normal rats. The ability of estrogen to increase the lactogen content of the pituitary was unimpaired when 100 International Units of estrone were administered daily with thiouracil for 21 days. When the rats received thiouracil for a two week period and were then given estrogen plus thiouracil for 10 days, the lactogenic hormone level was less than normal. Thirty-nine rats were used in this experiment. In a second experiment with 21 castrate male rats, the lactogen content of the pituitaries of rats treated with thiouracil plus stilbestrol increased as much as in those which received stilbestrol alone. Stilbestrol was injected at the rate of 10 micrograms daily for 21 days. The pituitary and thyroid weights of the thiouracil as well as the thiouracil plus estrogen treated rats were greater than the controls.—F.N.A.

NELSON, E. W., AND J. P. MICHAELS. Acute postpartum necrosis of the anterior hypophysis. *Am. J. Obst. and Gynec.* 52 (5): 817-825 (1946).

A 32 year old primigravida who had generalized convulsions and the typical picture of eclampsia was admitted to the hospital. The eclampsia was controlled and only two convulsions occurred. Onset of labor was spontaneous and delivery was uneventful. Blood loss was estimated at 400 cubic centimeters. However, the patient went into shock and remained at a severe shock level for one and one-half hours and mild shock level for nine hours despite intensive therapy. On the sixth hospital day, the picture of acute pituitary failure was present. The patient was controlled moderately well with 15 milligrams of desoxycorticosterone acetate daily, infusions and frequent use of hypertonic glucose. Thrombophlebitis, secondary to glucose administration, occurred on the 15th postpartum day and despite ligation, lumbar sympathetic block and general supportive measures, a staphylococcus albus septicemia occurred. Death occurred one month after delivery. Autopsy showed that 85 per cent of the anterior lobe of the hypophysis was replaced by a depressed yellow area. Microscopically, almost the entire anterior lobe stained pink without any cellular details. Only a few pyknotic remnants of nuclei were noted. Adjacent to the pars intermedia, a few acini were noted. The blood vessels

showed thrombosis and occlusion. The posterior lobe was normal. The adrenals were grossly normal but microscopically the cells of the reticular and fascicular layers were highly vacuolated and the medullary cells stained darker than usual.—*C.D.D.*

REID, D. E. Treatment of prolonged labor with posterior pituitary extract. *Am. J. Obst. and Gynec.* 52 (5): 719-734 (1946).

Posterior pituitary extract was administered to 1,699 patients who exhibited varying degrees of uterine inertia. The initial dose was usually one minim. An average total dose of 1 to 4 minims was given in the course of a single labor. The total dose never exceeded 15 minims. No deleterious effects were noted from the judicious use of the drug. There were no ruptured uteri. The author presents a good résumé of the arguments for this type of therapy.—*C.D.D.*

SPECK, G. Pregnancy in cases of pituitary dwarfism. *Am. J. Obst. and Gynec.* 51 (2): 217-220 (1946).

The author reports pregnancy in a 41 year old primigravid white single woman who was 42 inches tall. The patient was apparently classified as a pituitary dwarf on no particular objective basis of existing or previously existing hypopituitarism. The patient was delivered by Cesarean section. The infant lived 3 hours. An autopsy showed multiple congenital abnormalities, including meningocele, congenital cystic liver and polycystic kidneys.—*C.D.D.*

## THYROID

ERSHOFF, B. H. Effects of liver feeding on growth and ovarian development in the hyperthyroid rat. *Proc. Soc. Exper. Biol. and Med.* 64 (4): 500-503 (1947).

A total of 78 female rats averaging approximately 43 Gm. in weight were fed three different rations alone and supplemented with 0.5% U. S. P. desiccated thyroid for 60 days. The administration of liver completely counteracted the retardation of growth and inhibition of ovarian development observed in immature rats fed toxic amounts of thyroid. Wheat germ, yeast, or increased amounts of salt mixture, casein, thiamine, riboflavin, pyridoxine, calcium pantothenate, nicotinic acid, inositol, p-aminobenzoic acid, biotin or folic acid were ineffective in this regard. It is suggested that liver contains some factor other than the above required for normal growth and ovarian development in the immature thyroid-fed rat.—*F.N.A.*

SOFFER, L. J., M. VOLTERRA, J. L. GABRILOVE, A. POLLACK, AND MILDRED JACOBS. Effect of iodine and adrenalin on thyrotropin in Graves' disease and in normal and thyroidectomized dogs. *Proc. Soc. Exper. Biol. and Med.* 64 (4): 446-447 (1947).

The circulating thyrotropic hormone content of the blood serum of 13 patients with Graves' disease was studied by biological assay in the guinea pig. In all of these patients there was less circulating thyrotropic factor than is found in normal humans. Following lugolization the serum thyrotropin increased, reached a peak between the 4th and 6th days and then began to diminish. In 6 normal humans Lugol's solution produced but a barely perceptible increase in circulating thyrotropin. Subtotal thyroidectomy of pa-

tients with Graves' disease was followed by a further slight increase in blood thyrotropin. The injection of 1 cc. of adrenalin-in-oil twice daily in intact dogs produced marked hyperplasia of the thyroid by the 4th day of injection as shown by removal of one lobe of the gland. The further administration of adrenalin resulted in considerable decrease in the hyperplasia of the remaining lobe which was removed 10 days later. In totally thyroidectomized dogs adrenalin increased the circulating thyrotropic factor. A peak was reached in 4 to 6 days and diminished thereafter.—*F.N.A.*

(The concentration of adrenalin was not given.)

## ADRENALS

DAX, E. C., E. J. R. SMITH AND F. REITMAN. Adrenalectomy in mental disorder. *Brit. M. J.* 1: 215 (1947).

A case of adrenal virilism and one of adrenal pseudohermaphroditism, both with mental changes, were treated by unilateral adrenalectomy. The operation produced a drop in urinary steroids and the other biochemical changes which would be expected. In the case of adrenal virilism it also produced some physical change, but the mental picture was not materially improved in either patient. An unexplained persistent rise in the blood sedimentation rate followed operation in both cases.—*L.T.S.*

GUTMANN, D. Medullary suprarenal chromaffinoma producing malignant hypertension. *Brit. M. J.* 1: 563 (1947).

A case of chromaffinoma of the right adrenal medulla diagnosed during life is reported in a woman aged 38. Before operation could be performed the patient died from secondary malignant hypertension and uremia. The history of the case and the results of various examinations during and after the attacks are described. The tumor was not malignant.—*L.T.S.*

JAUDON, J. C. Hypofunction of the adrenals in early life. *J. Pediat.* 29: 696 (1946).

The author proposes that in many infants during their early weeks of life, there may be physiologic hypofunction of the adrenal glands. He believes that anorexia, failure to gain weight, unexplained tendency to dehydration, attacks of hypoglycemia and protracted diarrhea may very well be manifestations of low adreno-cortical function, particularly if they do not respond to treatment. These patients may be greatly benefited by hormone therapy. Detailed histories are presented in 9 cases which the author considers to have had low adrenal function. All of these patients were critically ill, and nearly all showed a dramatic improvement following the initiation of hormone therapy. Of the 8 patients receiving therapy, 7 lived; the 8th died from aspiration of milk. Of particular interest are the histories of identical premature twins each of whom developed convulsions followed by failure to gain weight, anorexia, loose stools and dehydration. The one, who was given no desoxycorticosterone acetate, died; the other, who received hormone therapy, recovered and has remained well! Most of the patients were observed for 3-10 weeks in the hospital without demonstrable improvement in their condition before hormone therapy was begun. The characteristics common to every infant described included general debility and weakness, appearance of impending shock and a marked tendency to dehydration in spite of adequate parenteral fluid therapy. In addition, 5 of the 9 patients had superimposed attacks of hypoglycemia which were imme-

diately relieved by elevation of the blood sugar. Two of the patients had no evidence of infection or gastro-intestinal disturbances; four patients had anorexia and loose stools from early days of life without evidence of infection; one patient had a transitory urinary infection without gastro-intestinal disturbances other than anorexia. Two patients with diarrhea developed staphylococcus septicemia readily responding to penicillin; the loose stools continued and the down-hill course persisted until hormone therapy was started. No harmful effects from the hormone injections were encountered. The author concludes that adreno-cortical hormone therapy may be a necessary adjunct in the maintenance of proper water and electrolyte balance, preservation of normal intestinal function, and the adjustment of a deranged carbohydrate metabolism in selected patients during early infancy. Hypoglycemia during early infancy is not a rare occurrence, and it is not uncommonly associated with symptoms of shock. The reappearance of severe hypoglycemia during early infancy appears to be prevented by the administration of pork adrenal-cortical extract. The author points out that during most of intra-uterine life, the true adrenal cortex remains a thin rim while the X- or fetal zone comprises the largest part of the gland and grows rapidly. During this stage of development, it is probable that the fetus does not rely entirely on the secretion of hormones from its own adrenal tissue for its rapid growth and development. Immediately following birth, the androgenic tissues involute and there is most likely an increased need for secretion of hormones in the true cortex; this in turn leads to enlargement of the true cortex during the first four weeks of life. During this adjustment period there may be a physiologically low level of activity of the true adrenal cortex. The author believes that this concept would explain the number of deaths in premature and full-term infants during the first few weeks of life which are preceded by unexplained vulnerability not responding to treatment.—*E.C.R., Jr.*

SPALDING, J. M. K. A case of phaeochromocytoma. *Brit. M. J.* 1: 442 (1947).

A case of phaeochromocytoma is described in a twenty-four year old woman showing the typical paroxysmal hypertension and associated symptoms. Operation was followed by a marked drop in blood pressure and shock, but adrenal extracts and salt solution given in large amounts carried her through this period. The convalescence was uneventful and fifteen months after the operation the patient was asymptomatic.—*L.T.S.*

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## SERUM GLUCURONIDASE ACTIVITY DURING NORMAL AND TOXEMIC PREGNANCY\*

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Gynecology, The University of Chicago, and the Chicago Lying-In Hospital*

THE introduction of a simple colorimetric method for the assay of mono- $\beta$ -glucuronidase activity (22) has made the present study possible. Increased urinary excretion of estriol and pregnandiol glucuronide in pregnancy (4, 14, 23) has stimulated interest in serum glucuronidase activity in this condition. The present work establishes the serum glucuronidase level of normal and pregnant women and shows how the toxemias of pregnancy can be enzymatically differentiated.

During pregnancy many changes occur in the enzymes of the blood. These are reported as an increase in protease (1), histaminase (2, 15, 25) and amylase (16) activity; and a decrease in lipase (19). In addition, extracts of the posterior lobe of the pituitary gland are inactivated by pregnancy serum (24, 27) or upon injection into pregnant patients (6). This antagonism can be assayed as "pitocinase" according to Page (18).

During pregnancy toxemia a basic enzymatic disturbance beyond the normal changes seems to occur. There is a decrease in certain proteolytic enzymes (7, 8, 12, 17); although fibrinolytic activity increases (21, 26).

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Serum histaminase (2) and pitocinase (18) values are scattered on both sides of the normal curve, and there is no apparent correlation between their levels and the severity of the disease. The organism is more reactive than normal to injection of extracts of posterior pituitary, particularly to its pressor and antidiuretic components (6). A decrease in serum cholinesterase (13) may occur.

#### MATERIAL AND METHOD

A total of 190 women were studied. Except for a few designated cases, these were obtained from the surgical and obstetrical services of the Albert Merritt Billings Hospital and the Chicago Lying-In Hospital. Clinically two principle types of pregnancy toxemia are recognized; those peculiar to pregnancy, commonly referred to as pre-eclampsia, and those developing independent of the pregnant state, usually called hypertensive toxemia. The differentiation of these groups depends largely upon certain clinical findings, as age, symptoms, weight gain, degree and persistence of hypertension, edema and proteinuria. Such criteria are obviously only relative, not absolute. In this study the diagnosis of toxemia, and differentiation as to the type, was made according to accepted standards (7). Cases were considered to be either pre-eclamptic, eclamptic or hypertensive toxemia.

Mono- $\beta$ -glucuronidase activity was determined by the method of Talalay, Fishman and Huggins (22), using 0.01 M. sodium-phenolphthalein glucuronide as substrate. The serum was obtained from clotted whole blood by centrifugation at high speed. Precautions to prevent hemolysis were taken, but this source of error was obviated by controls. Fresh tissues were thinly sliced, blotted to remove blood, and weighed. The weighed tissue was transferred to a chilled glass homogenizer, 5.0 cc. of distilled water were added, and the tissue was homogenized at high speed. After centrifugation, the supernatant was decanted and assayed in duplicate for glucuronidase activity. Results are expressed in micrograms of phenolphthalein liberated by 1.0 ml. of serum or by 1.0 Gm. wet weight of tissue in one hour under standard conditions of temperature and pH.

#### RESULTS AND INTERPRETATION

**1. Non-pregnant Controls:** In a group of 54 normal non-pregnant females varying in age from 2 to 60 years, the serum glucuronidase varied from 2.1 to 9.6 micrograms per ml. The mean and standard deviation for this group was  $5.3 \pm 2.1$  micrograms per ml.

**2. Normal pregnancy:** Figure 1 illustrates graphically the mean curve and maximum variation for serum glucuronidase from one hundred normal pregnant women. Activity is plotted against the weeks gestation calculated from the last menstrual period. A downward inclination of the mean curve

at its inception is apparent between 6 to 12 weeks when serum gonadotropins likewise fall. The subsequent rise in serum glucuronidase parallels that of serum estrogens (20). Furthermore, the mean curve for glucuronidase levels off from 38 to 40 weeks, as does that of serum estrogen. It is significant that estrogen (and progesterone) are partly excreted as glucuronides. In a series of 9 normal post-partum women the serum glucuronidase

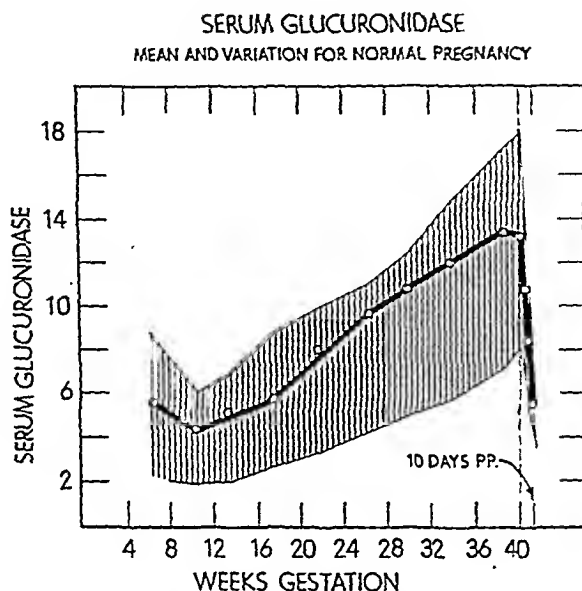


FIG. 1. Serum glucuronidase activity expressed in micrograms per ml. of one hundred normal pregnant women showing mean curve and variation.

dase level reached values less than 9.4 micrograms per ml. by the fifth to seventh post-partum day which is within the normal range for healthy controls.

**3. Toxemia of Pregnancy:** Figure 2 illustrates the serum glucuronidase activity in 24 cases of pregnancy toxemia as well as the relationship between these values and the normal mean curve. It becomes apparent that glucuronidase values in the 12 cases of hypertensive toxemia cluster about the mean glucuronidase curve, whereas those patients with pre-eclampsia (with two exceptions) have much higher values. In fact, a figure over 20 micrograms per ml. seems to indicate that the patient has pre-eclampsia. Of three patients with pre-eclampsia and one with eclampsia, three had reached normal values of serum glucuronidase by the eighth, eleventh and thirteenth post-partum days while one still had an elevated value upon discharge on the tenth day. Three normal patients who received 5 mg. of



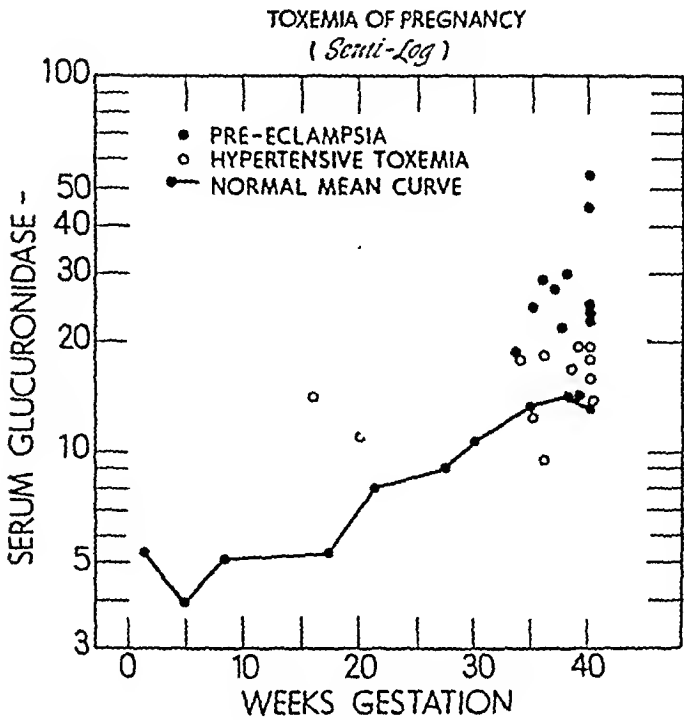


FIG. 2. Serum glucuronidase activity in micrograms per ml. in twenty-four cases of pregnancy toxemia. The mean curve of normal pregnancy (Fig. 1) is plotted for comparison.

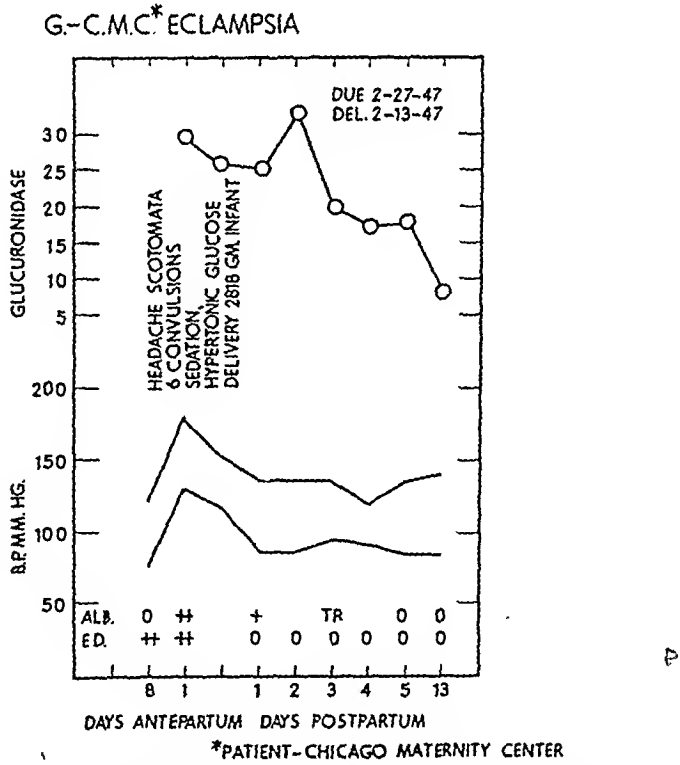


FIG. 3. Composite graph showing serum glucuronidase activity in micrograms per ml., systolic and diastolic blood pressure, albuminuria and edema during eclampsia.

diethyl stilbestrol daily during the first five post-partum days, to prevent lactation, continued to have elevated serum glucuronidase beyond the seventh post-partum day. The protocols for all patients with toxemia are given in Tables I and II. Figure 3 illustrates a composite graph for a patient with eclampsia showing the high glucuronidase levels in this syndrome with a decline to normal in thirteen days.

TABLE I. PRE-ECLAMPSIA

Identification	Age	Para	Gravida	Family History of Hypertension	Weeks Onset of Toxemia	Average Maximum Blood Pressure mm. Hg.	Average Maximum Proteinuria gm./24 hr.	Maximum Edema
339829	19	0	1	-	38	140/100	2.2	+
D.*	39	1	2	-	37	160/120	0.5	+
387436	25	0	1	+	40	186/90	9.1	+
387473	22	0	1	-	36	140/112	1.2	+
G.†	18	0	1	-	38	180/130	1.0	++
347493	23	0	1	-	37	156/100	1.3	++
383214	30	0	1	+	39	140/94	0.6	+
309066	35	6.	7	-	34	155/90	11.1	++++
390760	22	1	2	-	38	140/90	0.2	++
389897	20	0	1	-	36	174/120	1.5	+++
392846	18	0	1	-	34	156/100	0.2	+++
182062	31	0	1	-	33	130/80	0.27	++

\* Patient Lewis Memorial Hospital, Chicago, Illinois.

† Patient Chicago Maternity Center.

4. **Tissues:** The glucuronidase activity of 2 normal placentae was 1,772 and 2,185 micrograms per Gm. wet weight. The glucuronidase content of endometrium from a 7 and 40 week gestation was 580 and 360 micrograms per gram wet weight, respectively. Ovary and myometrium obtained at cesarean section of a 40 week gestation were 4,250 and 257 micrograms per gram. These values are low in comparison with liver, spleen and kidney from non-pregnant normal individuals, which were: kidney 6,850, liver 11,250 and spleen 6,500 micrograms per gram wet weight. This would indicate that the source of glucuronidase activity during pregnancy is not the gravid endometrium or the placenta.

5. **Infant and mother:** In a series of twelve comparative observations maternal and umbilical cord serum averaged 16.2 and 5.8 micrograms per

ml. respectively. In no case did the activity of cord blood equal or exceed that of the corresponding maternal blood. These observations exclude the fetus as a source of glucuronidase in the maternal serum; low values in the

TABLE II. HYPERTENSIVE TOXEMIA

Identifi- cation	Age	Para	Gravida	Family History of Hyper- tension	Weeks Onset of Toxemia	Average Maximum Blood Pressure mm. Hg.	Average Maxi- mum Protein- uria gm./24 hr.	Maximum Edema
399793	42	1	3	+	16	176/110	0	+
386821	31	3	4	+	10	166/96	0	0
389267	25	2	3	+	20	170/90	0	++
389272	28	0	1	+	36	156/106	0	+++
C.*	25	0	1	+	20	155/90	0	0
389847	32	0	1	+	16	190/110	0	+++
394045	31	0	1	-	37	180/130	0	++
385358	27	0	1	+	39	164/102	0.4	0
307733	21	0	1	-	38	148/90	0	0
2242	37	3	2	+	36	184/104	0	0
D.R.†	25	2	3	+	32	154/100	0.2	+
387437	23	0	1	-	39	176/118	0.2	++

\* Patient Roseland Community Hospital, Chicago, Illinois.

† Patient Lewis Memorial Hospital, Chicago, Illinois.

cord blood would indicate that the placenta was an effective barrier to the passage of the glucuronidase complex from maternal to fetal circulatory systems.

#### COMMENT

It is reported that the glucuronidase activity of the liver can be increased by administering borneol and menthol (10), and injection of estrogen increases the glucuronidase activity of the uterus (11). Moderately increased serum phenol levels during toxemia of pregnancy have been observed by Dieckmann (9). Glucuronidase probably regulates the conjugation of toxic substances having a free hydroxyl group with glucuronic acid (5) and thus increases their solubility and aids in their excretion by liver and kidney. However, the reason for the increased serum activity during pregnancy is not apparent. It would seem that the progressive increase of serum and urinary steroids (estrogens and pregnanediol)

during pregnancy would require a greater concentration of enzyme. On the other hand, the unusual increase of glucuronidase in the blood during pregnancy toxemia in the presence of a diminished serum and urine steroid content (22, 25) is puzzling, but may be compensatory. The evidence presented here lends support to those who suggest that pregnancy toxemia constitutes a basic enzymatic disturbance which is closely allied to estrogen, progesterone and gonadotropin metabolism. In addition study of serum glucuronidase seems to indicate that real differences between hypertensive toxemia and pre-eclampsia exist.

### CONCLUSIONS

1. Progressive increase of serum glucuronidase activity occurs during normal pregnancy.
2. Serum glucuronidase is increased during pre-eclampsia and eclampsia, but is not significantly increased in hypertensive toxemia.
3. Determination of serum glucuronidase may be of value in the diagnosis of pre-eclampsia and eclampsia.
4. The source of the increased glucuronidase activity in the serum of pregnant women is unknown.

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# RELATION OF URINARY STEROIDS TO THE DIAGNOSIS OF ADRENAL CORTICAL TUMORS AND ADRENAL CORTICAL HYPERPLASIA: QUANTITATIVE AND ISOLATION STUDIES

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CLINICAL pictures which accompany functioning tumors of the adrenal cortex are neither specific nor diagnostic. Other pathologic conditions produce similar or even identical symptoms. Only one of these other conditions need be mentioned here; namely, adrenal cortical hyperplasia.\* For therapeutic reasons it is very important to distinguish cases of adrenal cortical tumor from adrenal cortical hyperplasia and from the other clinical and pathologic entities which simulate both of them. The discussion that follows centers around the chemical and biologic methods that can be employed to help make these distinctions.

Urine of patients who have functioning adrenal cortical tumors usually contains excessive amounts of both androgens and 17-ketosteroids. The former can be determined only by biologic methods, whereas the latter, which include not only androgenic steroids but others which seem to be biologically inert, can be determined by chemical methods. Kenyon, Gallagher, Peterson, Dorfman and Koch (1937) (27) were among the first to demonstrate that there may be large amounts of androgenic material in the urine excreted by patients having these tumors. They studied fifteen cases of "virilism" among women. Twelve patients excreted normal amounts of androgens, two excreted 25 per cent more than normal and one, who had a carcinoma of the adrenal cortex, excreted very large amounts. Similar observations were made by R. K. Callow (1938) (11). He determined both the androgen and the 17-ketosteroid content of the urine. In his series of cases there was one woman who had Cushing's syndrome, also a girl aged nineteen years who had an adrenal cortical tumor,

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\* Detailed descriptions of the clinical symptoms which accompany adrenal cortical tumors and hyperplasia may be found in reviews by Haymaker and Anderson (1938) (25), by Kepler and Keating (1941) (28), by Cahill (1944) (7), by del Castillo (1941) (12) and by Kenyon (1944) (26).

The term "hyperplasia" is used here in a loose clinicopathologic sense to denote bilaterally enlarged hyperfunctioning adrenal cortices associated with such clinical pictures as pseudohermaphroditism, adrenal virilism, Cushing's syndrome and others which also may occur in conjunction with adrenal cortical tumor.

and several women who were virilized but who presumably did not have adrenal cortical tumor. Only in the case of adrenal tumor was there found an increase of the urinary content of both androgenic and 17-ketosteroidal material. Callow therefore came to the conclusion that "adrenal tumor can be diagnosed with the aid of androgen assay." Levy Simpson (1938) (31), however, in a discussion of Callow's paper, pointed out that Callow's conclusion should be qualified since, in Levy Simpson's experience, the urine of patients having adrenal cortical hyperplasia sometimes contained amounts of androgenic material comparable to those obtained in cases of adrenal cortical tumor.

Shortly before these developments Frank (1934, 1937) (18, 19) made the astounding and seemingly paradoxical discovery that the estrogen content of the urine was increased in four cases of adrenal cortical tumor. From these observations he concluded that in the absence of pregnancy the presence of 500 to 1,000 mouse units of estrogenic substance per liter of urine should be considered strong evidence in favor of adrenal carcinoma. Frank's observation was confirmed in isolated instances in which the patients were women reported by Graef, Bunim and Rottino (1936) (24), by Lukens and Palmer (1940) (33), by Cahill, Loeb, Kurzrok, Stout and Smith (1936) (8) and by McGavack (1940) (34) and in a case in which the patient was a feminized man reported by Levy Simpson and Joll (1938) (32). However, it soon became apparent from the case reports of Walters and Kepler (1938) (48), of Dorfman, Wilson and Peters (1940) (15) of Crooke and R. K. Callow (1939) (13) and of Bruins Slot (1936) (4) that a functioning adrenal cortical tumor of women usually was not associated with the urinary excretion of large amounts of estrogenic material.

Knowing that the urine of patients having adrenal cortical tumors contained androgens, investigators naturally tried to isolate the specific compounds which might be responsible for the androgenic activity. Some of the early work along this line was done by Crooke and R. K. Callow (1939) (13). Four cases of Cushing's syndrome were investigated. In two of these an adrenal cortical tumor was responsible for the clinical picture. In the other two cases, in which adrenal cortical tumor was excluded, the excretion of both androgens and 17-ketosteroids was found to be relatively normal. In the former it was shown that the excretion of both of these groups of substances was increased and that a specific compound, dehydroisoandrosterone, was largely responsible for the increased excretion of 17-ketosteroids. Subsequently, these observations were confirmed. Talbot, Butler and MacLachlan (1940) (43) Fraser, Forbes, Albright, Sul-kowitch and Reifenstein (1941) (20) Patterson, McPhee and Greenwood (1942) (40) and Friedgood and Whidden (1939) (22) verified the increased excretion of 17-ketosteroids and Wolfe, Fieser and Friedgood (1941) (50)

Warren (1945) (49) and N. H. Callow and Crooke<sup>1</sup> (1944) (9) verified the observation that the excretion of dehydroisoandrosterone was increased.

As experience with the determination of the urinary 17-ketosteroids increased it soon became evident that increased values were not pathognomonic for adrenal cortical tumor. To increase the specificity of the test, Talbot, Butler and MacLachlan (1940) (43) devised a method for the determination of the 3( $\beta$ )-alcoholic fraction of these substances. This fraction may be made up of several 3( $\beta$ )-alcoholic steroids, among which is the compound previously mentioned, dehydroisoandrosterone. They found that the 3( $\beta$ )-alcoholic fraction formed not more than 10 per cent of the 17-ketosteroids for normal men, women and children, while it accounted for 50 and 63 per cent of the total in two cases of adrenal tumor. In a later study of four cases of adrenal tumor, Talbot, Butler and Berman (1942) (42) found 3( $\beta$ )-alcoholic fractions of 22, 49, 65 and 38 per cent of the total 17-ketosteroids. In Friedgood's (1944) (21) experience cortical hyperplasia was associated with a 3( $\beta$ )-alcoholic fraction of less than 25 per cent and cortical tumors (three cases) were associated with 3( $\beta$ )-alcoholic fractions of 30 to 70 per cent of the total 17-ketosteroids.

On the basis of work done by Butler and Marrian (1937, 1938) (5, 6), Broster and Vines (1937) (3) suggested that the presence of the compound pregnane-3( $\alpha$ ),17,20-triol in urine was a phenomenon specific for virilism of adrenal origin. Butler and Marrian felt that the suggestion of Broster and Vines was justified since the compound had been found only in the urine of patients having adrenal virilism (two cases of adrenal hyperplasia).

Recently, Anderson, Hain and Patterson (1943) (2) described a case of adrenal tumor which was associated with an abnormally high excretion of pregnanediol (12 to 20 mg. per day) as well as large amounts of 17-ketosteroids (215 mg. on one day). Pregnan-3( $\alpha$ ),17,20-triol was not found. They inferred that a diagnosis of adrenal tumor or of adrenal hyperplasia could be made on the basis of the pregnanediol output alone. Venn- ing, Weil and Browne (1939) (47) and Salmon, Geist and Salmon (1941) (41) also isolated sodium pregnanediol glucuronidate from the urine of patients who had adrenal carcinoma and of patients who had adrenal hyperplasia. Furthermore, Genitis and Bronstein (1942) (23) reported data on two female pseudohermaphrodites, four and ten years old, who excreted as much as 20 mg. of pregnanediol in forty-eight hours. The excretions of 17-ketosteroids were 27 mg. and 30 to 69 mg. in twenty-four hours. However, Talbot, Butler and Berman (1942) (42) found that the pregnanediol values were not consistently elevated in patients having adrenal cortical hyperplasia.

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<sup>1</sup> Callow and Crooke (1944) (9) reported one case of adrenal cortical tumor in which the excretion of 17-ketosteroids was not increased.



In brief, the literature shows clearly that the determination of the urinary estrogens, androgens and 17-ketosteroids is a valuable tool in the recognition of adrenal cortical hyperplasia and functioning adrenal cortical tumor and that these two conditions frequently can be distinguished one from another by determining the proportion of the 3( $\beta$ )-alcoholic 17-ketosteroids. It is also clear that pregnanediol may be excreted in the urine by patients having either adrenal cortical tumor or adrenal cortical hyperplasia and that pregnane-3( $\alpha$ ),17,20-triol may be present in the urine of patients suffering from adrenal virilism associated with adrenal cortical hyperplasia. On the other hand, it is evident that excessive amounts of urinary androgens, estrogens and 17-ketosteroids are not invariably present in the urine in cases of adrenal cortical tumor, that the differential diagnosis of adrenal cortical tumor and adrenal cortical hyperplasia cannot always be made by determining the androgenic content of the urine and that adrenal cortical hyperplasia is not necessarily characterized by the presence of pregnanediol in the urine. Finally, it is evident that investigators have been handicapped by having only small series of cases and sometimes have been inclined to infer too much from too little.

The report that follows deals with data that we have collected from patients having either adrenal cortical tumors or adrenal cortical hyperplasia.

#### MATERIAL AND METHODS

Details of the clinical records will be submitted for publication in a separate report. A summary of the significant abnormalities is presented in table 1. In tabulating the cases we attempted to follow the classification recently proposed by Kenyon (1944) (26). His proposals were modified slightly so as to include not only the neoplasms but also the so-called hyperplastic lesions:

Clinical pictures associated with hyperfunctioning adrenal cortical lesions.

1. Adrenogenital syndrome (including pseudohermaphroditism).
2. Cushing's syndrome.
3. Intergrades between types 1 and 2.
4. Isolated expressions of the lesion.
5. Feminization.
6. Lesions without endocrine manifestations.

For various reasons the practical application of this classification, or for that matter any other, is often very difficult when one is dealing with specific cases. We have discussed this problem in a recent paper which was

TABLE 1. CLINICAL OBSERVATIONS

Case	Age, years	Sex	Pathologic lesion*	Blood pressure, mm. of mercury	Diabetes, requiring insulin	Osteoporosis	Weakness	Striae	Hirsutism	Acanthosis	Enlarged clitoris	Florid color	Obesity	Glucose tolerance curve†	Remarks
3	63	F	T	162/88		+	+	-	+	-	-	+	-	92, 194, 208, 102	Predominantly Cushing's syndrome
5	25	F	T	204/115		+	+	+	+	-	-	+	-	77, 112, 154, 104	Predominantly Cushing's syndrome
6	3	F	T	110/80		-	-	-	-	+	+	+	-		Adrenogenital syndrome with somatic and heterologous sexual precocity
7	27	F	T	118/68		-	+	-	+	-	-	-	-	71, 109, 176, 78	Isolated endocrine symptoms
8	45	F	T	225		-	-	-	+	-	+	+	-	85, 118, 208, 149	Adrenogenital syndrome
9	3	F	H	110/68		-	-	-	-	-	+	-	-		Feminine pseudohermaphrodite with pubertas praecox
10	5	F	H	100/60		-	-	-	-	-	+	-	-		Feminine pseudohermaphrodite with pubertas praecox
11	10	F	H	110/80		-	-	-	+	-	+	-	-		Feminine pseudohermaphrodite
12	25	F	T	160/140		-	+	+	+	-	+	-	-	"Diabetic" type	Predominantly Cushing's syndrome with features of the adrenogenital syndrome
13	9	M	H	120/70		-	-	-	-	-	-	-	-		Homologous pubertas praecox Adrenogenital syndrome (?)
14	53	F	T	160/84		+	+	-	+	+	+	+	-		Predominantly Cushing's syndrome
15	55	F	T	100/60					+				-		Incompletely studied; metastasis present
16	40	F	T	154/92		-	-	+	+	+	+	+	+		Predominantly Cushing's syndrome
17	39	M	T	145/90	++	-	-	-		-	-	-	-		Nonfunctioning (?) neoplasm
18	32	M	T	110/72			-	-		-	-	-	-	86, 107, 90, 83	No clinical endocrine abnormalities but increased excretion of 17-ketosteroids
19	10	F	H	120/80		-	-	-	-	-	+	-	-		Feminine pseudohermaphrodite
20	27	F	H	160/76	+	-	+	-	-	+	-	+	+		Cushing's syndrome
21	29	M	H	175/135		+	-	-	-	-	-	+	+		Cushing's syndrome
22	26	F	H	150/105		-	+	+	+	-	-	+	-		Cushing's syndrome

\* H = hyperplasia; T = tumor.

† Blood glucose in mg. per 100 c.c. The values were obtained, in the order given, before and ½ hour, 2 hours and 3 hours after administration of glucose.

‡ Diabetes was present after removal of the tumor but in milder degree than before removal.

presented at the 1946 Laurentian Hormone Conference (Kepler, et al. 1946) (29).

The diagnosis in all but two instances was established by surgical ex-

ploration. These two cases deserve brief comment. One patient (case 12), aged twenty-five years, had acquired symptoms of an adrenal cortical tumor at the age of eighteen years. Her habitus was not unlike that of an achondroplastic dwarf. In addition, she had many of the features of Cushing's syndrome but the features of virilism were more marked than those usually encountered in this clinical picture. In the upper right quadrant of the abdomen there was a large tumor which had its origin above the right kidney and there were shadows in the roentgenograms of the lungs which had the characteristics of metastatic malignant lesions. Under the circumstances there seemed to be no point in establishing the diagnosis surgically. One can be reasonably certain, however, that the patient did have an adrenal cortical tumor but the diagnosis cannot be regarded as proved. The other patient was a boy with homologous sexual precocity. His sister, the patient in case 9, had a similar disorder but the sexual precocity was heterologous in type. Her adrenal glands were explored surgically and found to be hyperplastic. It is likely, therefore, that the adrenal glands in the boy's case were hyperplastic, although here again the diagnosis cannot be regarded as proved.

The specimens of urine for quantitative determinations were collected in brown bottles containing 5 ml. of concentrated hydrochloric acid. The fresh specimens were boiled for ten minutes after addition of 0.1 volume of concentrated hydrochloric acid and then were extracted with carbon tetrachloride. The extracts were partially purified by the procedure of Talbot, Butler, MacLachlan and Jones (1940) (45). The method of N. H. Callow and associates (1938) (10) was used for determination of the total 17-ketosteroids with application of a correction equation to compensate for overestimation due to nonketonic chromogens (Engstrom and Mason, 1943 (16)). A modification of the method of Talbot, Butler and MacLachlan (1940) (43) was used for the determination of the 3( $\beta$ )-alcoholic 17-ketosteroid fraction.

The procedures used for isolation of the various steroids have been described elsewhere (Mason and Kepler, 1945) (37).

## RESULTS

With Engstrom (1944) (17) in a previous paper we have presented quantitative data on the urinary excretion of 17-ketosteroids in eight cases of proved adrenal cortical tumors and three cases of cortical hyperplasia. Eight of these cases are included in the present studies and have been assigned the same numbers used in the previous report. In table 2 are presented further quantitative data on cases 5 and 11, together with data obtained in five additional cases of tumor and four of hyperplasia. The condition of the adrenal glands was established by surgical exploration in

all of the cases presented in table 2. The patients in cases 11 and 19 were pseudohermaphrodites. The patients in cases 20, 21, and 22 had frank, easily recognized Cushing's syndrome. In the light of the diagnostic criteria proposed by Crooke and R. K. Callow (1939) (13) and by Talbot and his associates (1940) (44) and mentioned previously in this report, cases 16 and 17 are particularly interesting. One of these patients (case

TABLE 2. THE 17-KETOSTEROID EXCRETION OF PATIENTS WHO HAD  
ADRENAL CORTICAL TUMORS OR HYPERPLASIA

Case	Age, years	Sex	Specimens collected	Average 17-ketosteroid excretion per 24 hrs., mg.	$\beta$ -Fraction, average per cent of total 17-keto- steroids
Adrenal cortical tumor					
5	26	F	3	107*	60
14	53	F	1	800	69
15	55	F	1	1,005	77
16	40	F	2	30	7.5
17	39	M	2	4.8	—
18	32	M	2	68	48
Adrenal cortical hyperplasia†					
11	21	F	1	123‡	20
19	10	F	2	31	14
20	27	F	3	24	14
21	29	M	2	28	2
22	26	F	2	10	5

\* A preoperative value of 130 mg. for this case was reported previously. The above value was obtained after recurrence of the tumor approximately one year later.

† In cases 11 and 19 the patients were female pseudohermaphrodites with masculine characteristics. In cases 20, 21 and 22 the patients had Cushing's syndrome.

‡ A value of 75.2 mg. was reported previously. The above value was obtained approximately two years later.

16) had a functioning adrenal cortical tumor. The value for the total excretion of 17-ketosteroids per twenty-four hours (30 mg.), although well above the normal range, was not greatly elevated and was within the range found in one previous case and in three of the present cases of hyperplasia. The 3( $\beta$ )-alcoholic fraction of the 17-ketosteroids was well within normal limits. In case 17 the tumor probably was not functioning. The value of 4.8 mg. for the total 17-ketosteroids was definitely less than nor-

mal. Our lowest normal value for a man is 6.0 mg. Because of the low value the 3( $\beta$ )-alcoholic fraction was not determined in case 17.

There is already evidence in the literature that occasionally an adrenal cortical tumor may be present without a significant increase of the urinary excretion of androgens or 17-ketosteroids. Lawrence (1937) (30) described a case in which the excretion of androgens was only 1 to 2 capon units in twenty-four hours. Albright<sup>2</sup> (1943) (1) referred to two cases with proved tumors in which the excretion of 17-ketosteroids was 13.7 and 10.2 to 11.1 mg. in twenty-four hours and N. H. Callow and Crooke (1944) (9) reported one case of a woman sixty-one years of age who excreted 14.5 to 20 mg. per day.

In the five cases of hyperplasia one value for the 17-ketosteroids was normal, three were moderately elevated and one was greatly increased. The present value for case 11 represents an increase of 64 per cent in the amount excreted as compared to the value obtained two years previously. The 3( $\beta$ )-alcoholic fraction of the 17-ketosteroids of the group of cases with hyperplasia comprised 2 to 20 per cent of the total.

Table 2 reveals that, with two exceptions, a consideration of both the total and the 3( $\beta$ )-alcoholic fraction of the 17-ketosteroids distinguishes cortical tumors from hyperplasia. Case 16 could not be differentiated from hyperplasia on this basis. Determination of the 17-ketosteroids was sufficiently high to suggest the presence of a tumor but the 3( $\beta$ )-alcoholic fraction was only moderately increased.

Table 3 summarizes the results of the isolation of steroids from the urine in six cases of cortical tumor and four of hyperplasia. A detailed account of the chemical procedures and substances isolated has been given elsewhere (Mason and Kepler, 1945) (37). Because of wide variations in the amounts of urine available and changes in the procedures used, the quantities given are of doubtful significance for comparison of the steroid excretion in one case with that in another. However, when possible, the values have been calculated in terms of the amount per day and included in the table in parentheses.

Deydroisoandrosterone was obtained in large amounts from all but one of the cases of tumor. In each instance the amounts isolated were greater than the sum of the amounts of the other ketones isolated. This result further substantiates the suggestion of Crooke and R. K. Callow (1939) (13) that cortical tumors are usually accompanied by the excretion of excessive amounts of this substance. On the other hand the amount of

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<sup>2</sup> Recently Albright (personal communication) has had two additional cases in which the values were normal, and we also have recently had a case of this type.

dehydroisoandrosterone that could be isolated in the cases of hyperplasia was a small fraction of the ketones isolated. In one case none could be found. Case 12 was exceptional among the cases of tumor in that no dehydroisoandrosterone could be isolated. A 17-ketosteroid determination was not made but a bio-assay for androgens indicated the presence of the equivalent of not more than 5 mg. of androsterone in the urine excreted during a twenty-four hour period.

TABLE 3. SUMMARY OF THE STEROID FRACTIONS ISOLATED FROM THE URINE OF PATIENTS WITH ADRENAL CORTICAL TUMORS OR HYPERPLASIA

Case	Urine			Substances isolated									
	Age, years	Volume, liters	Days	Neutral extract			Ketones				Nonketones		
				Total wt., mg.	Ketonic fraction, mg.	Nonketonic fraction, mg.	Dehydroisoandrosterone, mg.	Androsterone, mg.	17-one, mg.	Etiocholan-3(α)-ol-17-one, mg.	Androstane-3(α)-11-diol-17-one, mg.	Estione, mg.	Pregmane-3(α)-20(α)-diol, mg.
													Pregmane-3(β)-20(β)-diol, mg.
Adrenal cortical tumors													
3	63		14	1,450 (104)*	570 (39.3)	6,400 (457)	184 (13.1)		65 (4.6)	30 (2.1)		28 (2.0)	
5	25†	43.0	26	9,300 (358)	5,600 (216)	2,900 (112)	2,190 (84.3)	49 (1.9)	192 (7.4)	11 (0.4)	62 (2.4)	107 (4.1)	
6	3	6.4	13	1,430 (110)	790 (60.8)	710 (54.6)	1,525 (117.3)		33 (2.5)			53 (4.1)	
7	27	5.7	3	1,570 (523)	870 (290)		391 (130.3)		13 (4.3)			13 (4.3)	
8	45	50.4	31	8,000 (258)	3,500 (113)	3,000 (96.8)	740 (23.8)	290 (9.2)	127 (4.1)	77 (2.5)		90 (2.9)	
12	25	15.0	13	3,200 (246)	1,250 (96.2)	1,630 (125.3)		55 (4.2)				127 (9.8)	47 (3.6)
Adrenal cortical hyperplasia													
9	3	18.0		1,200	320	590	33‡	55‡		65‡			27
13	9	18.0		1,160	520	470						Trace	35
10	5	11.0	13	1,350 (104)	410 (31.5)	410 (31.5)		19 (1.5)	11 (0.8)	23 (1.8)		71 (5.5)	
11	19†	54.0		4,480	2,010	1,650	7	122		80		210	74

\* The values in parentheses are those calculated as the amounts obtained per day.

† The data given in this table were obtained before the data in table 2, hence the difference in the ages in cases 5 and 11 in the two tables.

‡ The ketonic fractions in cases 9 and 13 were combined by mistake and therefore were worked up together.

Androsterone was found consistently in the urine in the cases of hyperplasia but in only three of the cases of tumor. Etiocholan-3( $\alpha$ )-ol-17-one was isolated in all of the cases of tumor in which dehydroisoandrosterone was found but in only one case of hyperplasia. The significance of these variations is not apparent but the results are in general agreement with the quantitative results of Dobriner and his associates (1942) (14). In their one case of adrenal tumor the urine contained androsterone while in two of their cases of hyperplasia very little etiocholan-3( $\alpha$ )-ol-17-one could be found. Recent studies (Mason and Kepler, 1945, 1947) (36, 38) of the metabolism of dehydroisoandrosterone suggest that the variations may be due to individual differences in the conversion of this substance to androsterone and etiocholan-3( $\alpha$ )-ol-17-one.

Androstane-3( $\alpha$ ),11-diol-17-one (11-hydroxyandrosterone) is a recently discovered compound (Mason and Kepler, 1945). It has been found recently (Mason, 1946) (35) also in normal human male urine to the extent of 0.3 mg. per liter. It was found to be present in considerably increased amounts in most of these cases of adrenal cortical disease. Miller, Dorfman and Sevringhaus (1946) (39) have recently isolated very large amounts (10 mg. per liter) of this substance from the urine of a woman who had an adrenal tumor. Presumably it is a metabolic product of some of the adrenal steroids which have a hydroxyl group at carbon number eleven. Corticosterone, 17-hydroxycorticosterone and related hormones suggest themselves as likely precursors. If so, an increased production of these cortical hormones by the tumors and hyperplastic cortices is indicated.

Although Broster and Vines (1937) (3) and Butler and Marrian (1938) (6) have emphasized the relation of pregnane-3( $\alpha$ ),17,20-triol to adrenal cortical hyperplasia it is reasonable to suspect that it would also occur in the urine of patients who have cortical tumors. It was found in three cases of hyperplasia and one case of tumor. Elution of the chromatogram in case 6 was not carried far enough to obtain this substance if present. It definitely was not found in the urine in cases 3, 5, 7 and 8 and previous experience makes it fairly certain that had it been present in appreciable amounts it would have been found. It is not known how easily this pregnanetriol is destroyed by the hydrolysis of the urine but preliminary experiments with a mixture of sodium pregnanediol and pregnanetriol glucuronidates which was isolated from the urine in case 11 indicate that pregnane-3( $\alpha$ ), 17, 20-triol is more sensitive to the hydrolytic procedure than pregnanediol.

The inference of Anderson, Hain and Patterson (1943) (2) that a diagnosis of adrenal tumor or of adrenal hyperplasia could be made on the basis of the output of pregnanediol receives support from our results except for

the brother and sister who had hyperplasia (cases 9 and 13). There was, however, in the urine of the boy (case 13) an isomer of the usual pregnanediol (pregnane-3( $\alpha$ ),20( $\alpha$ )-diol). The isomer had the properties of pregnane-3( $\beta$ ),20( $\alpha$ )-diol. It was also present in the urine in case 11.

Although pregnane-3( $\alpha$ ),20( $\alpha$ )-diol was isolated after hydrolysis it could not always be obtained as sodium pregnanediol glucuronide by the usual procedure of Venning (1937) (46). This procedure, with some modifications required by the large amount of material in the butyl alcohol extract, was used for cases 3, 5, 6 and 8 of adrenal tumors and for case 11 of hyperplasia. Sodium pregnanediol glucuronide was isolated in cases 3, 6 and 11. The amount in case 6 was only a trace of crystals which after two recrystallizations had the correct appearance but the rather low melting point of 252–253°. Venning gave a melting point of 268–271° with decomposition. The glucuronide obtained from case 3 corresponded to 5 and 13 mg. of pregnanediol in two determinations. The pregnanediol glucuronide obtained in case 11 was accompanied by a glucuronide which gave pregnane-3( $\alpha$ ),17,20-triol on hydrolysis. It was not possible to separate the two glucuronides by recrystallization. A crystalline precipitate which was obtained in case 8 when sodium pregnanediol glucuronide was sought was identified as sodium dehydroisoandrosterone sulfate, which was later obtained in large amounts from this urine. A crystalline precipitate could not be obtained at the proper point from the urine in case 5 even though a relatively large amount of pregnanediol was isolated after hydrolysis of the urine.

Although, as has been mentioned elsewhere, Frank (1934, 1937) (18, 19) described two consecutive cases and later two more of adrenal tumor with greatly elevated excretions of estrogens, in only one of our cases was an abnormal amount excreted. One assay of the urine in case 5 gave a value of 9,000 rat units in twenty-four hours. Assay of the extract of the pooled urine gave an average value of 3,400 rat units for twenty-four hours or a total for twenty-six days of 88,400 rat units, which is equivalent approximately to 177 mg. of estrone. Actually, 62 mg. of estrone (m. p. 247–253°) were isolated. Recrystallization raised the melting point to 256°. The melting point was not depressed by admixture with authentic estrone. Although crystals were obtained from the fractions which may have contained estradiol and estriol, the melting point of the crystals did not correspond to that of either of these substances.

#### COMMENT

Consideration of the data presented shows that there is no single chemical criterion by which an adrenal cortical tumor can always be differen-



tiated from cortical hyperplasia. The most valuable contributions of the laboratory are determinations of the total 17-ketosteroids and of the 3( $\beta$ )-alcoholic fraction. These two determinations usually help to distinguish between a tumor and hyperplasia. However, it is possible for a functioning adrenal tumor (case 16) to be associated with a value for the 17-ketosteroids that is only two to three times the average normal value and with a 3( $\beta$ )-alcoholic fraction that is not increased beyond the normal range of values. Such a combination of results would ordinarily be interpreted as indicative of cortical hyperplasia.

Assay of the urine for estrogens may occasionally indicate the presence of a tumor. No cases have been reported in which excessive excretion of estrogens was associated with cortical hyperplasia.

Very little attention has been paid to the nonketonic fraction. A determination of pregnanediol or of 17,20-dihydroxy compounds such as pregnane-3( $\alpha$ ),17,20-triol may well be informative in some instances. Unfortunately our results indicate that, although pregnanetriol is found more often in association with hyperplasia, it may occasionally be found in association with a tumor. Increased amounts of pregnanediol may be found in cases of tumor and of hyperplasia. In this group of cases it was present more consistently in the urine in cases of tumor than in cases of hyperplasia.

#### SUMMARY

The urinary 17-ketosteroids and the 3( $\beta$ )-alcoholic fraction of the 17-ketosteroids were determined in six cases of adrenal cortical tumor and five cases of cortical hyperplasia. The ketonic and nonketonic steroids were isolated from the urine in six cases of tumor and four of hyperplasia.

In one case of tumor the quantitative determination of 17-ketosteroids gave a result below the normal range of values. In another case, the 3( $\beta$ )-alcoholic fraction was only 7.5 per cent of the total 17-ketosteroids (30 mg.). In the four other cases of tumor the 3( $\beta$ )-alcoholic fraction was 48 to 77 per cent of the total amounts (68 to 1,005 mg.) of 17-ketosteroids. In cases of hyperplasia the 3( $\beta$ )-alcoholic fraction was 2 to 20 per cent of the total 17-ketosteroids (10 to 123 mg.). Consideration of these results in conjunction with those of other investigators indicates that excretion of 50 mg. or more of 17-ketosteroids per day with a 3( $\beta$ )-alcoholic fraction of 50 per cent or more is strong evidence in favor of the presence of adrenal tumor. In a few instances, however, an adrenal tumor may be present when these values are considerably lower and within the range of values which have been found in association with cortical hyperplasia.

The results of the isolation studies emphasize again the relatively large

amounts of dehydroisoandrosterone which are excreted in cases of adrenal tumor. Very little of this substance was isolated in the cases of hyperplasia. Androsterone and etiocholan-3( $\alpha$ )-ol-17-one were isolated, the latter more consistently in the cases of tumor, the former in all cases of hyperplasia. Pregnane-3( $\alpha$ ),20( $\alpha$ )-diol was isolated in all cases of tumor and in three cases of hyperplasia. Pregnane-3( $\beta$ ),20( $\alpha$ )-diol was tentatively identified in two cases of hyperplasia. Pregnane-3( $\alpha$ ),17,20-triol was found in one case of tumor and in three cases of hyperplasia. A new 17-ketosteroid, androstane-3( $\alpha$ ),11-diol-17-one, was isolated in three cases of tumor and in all of the cases of hyperplasia. It appears to be related to the 11-oxygenated adrenal hormones. Excessive excretion of estrogens was observed in only one case (of tumor) and estrone was isolated from the urine in this case.

Pregnane-3( $\alpha$ ),17,20-triol appears to be found more often in association with cortical hyperplasia than with tumors. Examination of the urine for this substance may well be of value for confirmation of a diagnosis of cortical hyperplasia.

Finally, our observations, taken in conjunction with others reported in the literature, indicate that every patient presenting symptoms suggesting adrenal cortical tumor or adrenal cortical hyperplasia must be studied individually, using both clinical and laboratory procedures. Neither is self-sufficient.

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# PLASMA PROTEIN PATTERN (TISELIUS ELECTROPHORETIC TECHNIQUE) IN CUSHING'S SYNDROME

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DOUGHERTY and White (7) reported an increase of the serum  $\gamma$ -globulin in rabbits following injection of large amounts of adrenal extract, and a release of  $\gamma$ -globulin antibody from lymphoid tissue following adrenal stimulation by adrenotrophic hormone, or following injection of adrenal extract (2). These observations suggested the question of what changes, if any, occur in the  $\gamma$ -globulin level of the plasma of patients with hyperadrenal function of the Cushing's syndrome type.

## METHODS

A group of 9 patients proved by physical examination and pertinent laboratory findings to have Cushing's syndrome were studied. The results were contrasted with those obtained on patients with Addison's disease (5) and hypo-adrenal function of pituitary origin (6). In 8 of the patients with Cushing's syndrome, the plasma proteins were studied before treatment was instituted. Three of this group were observed for a period of as long as three years after therapy. One patient, no. 9, who had had a partial adrenalectomy and x-ray therapy to the pituitary gland, was studied five and a half years after treatment. He was in excellent condition at the time of the study. The plasma protein patterns of 4 patients with acromegaly were also studied.

The Tiselius electrophoretic technique as modified by Longsworth was employed. The method of calculating the results and the normal values obtained in this laboratory have been given in detail (3).

## RESULTS

The data obtained on the 8 patients who showed evidence of adrenal cortical hyperactivity as judged by the presence of clinical signs of Cushing's syndrome, is summarized in Fig. 1. In all cases the plasma  $\gamma$ -globulin level was decreased below normal. The  $\alpha_2$ -globulin was above the highest normal level in 3 cases and above the average normal value in all cases, and the albumin was decreased in 7. There was no significant change in the total protein level, the  $\beta$ -globulin or fibrinogen.

Three of the eight patients, and one (no. 9) on whom no pretreatment picture was obtained were given x-ray irradiation to the pituitary gland.

Hemiadrenalectomy of both adrenal glands was also carried out on these patients. The plasma protein picture (no. 2, 3, and 6) did not return to normal, although there was some increase in the  $\gamma$ -globulin level in no. 2 and no. 6 (Table 1). The plasma protein pattern of case 9 varied only slightly from normal. It is interesting that the clinical condition of this patient was also essentially normal at the time the study was made.

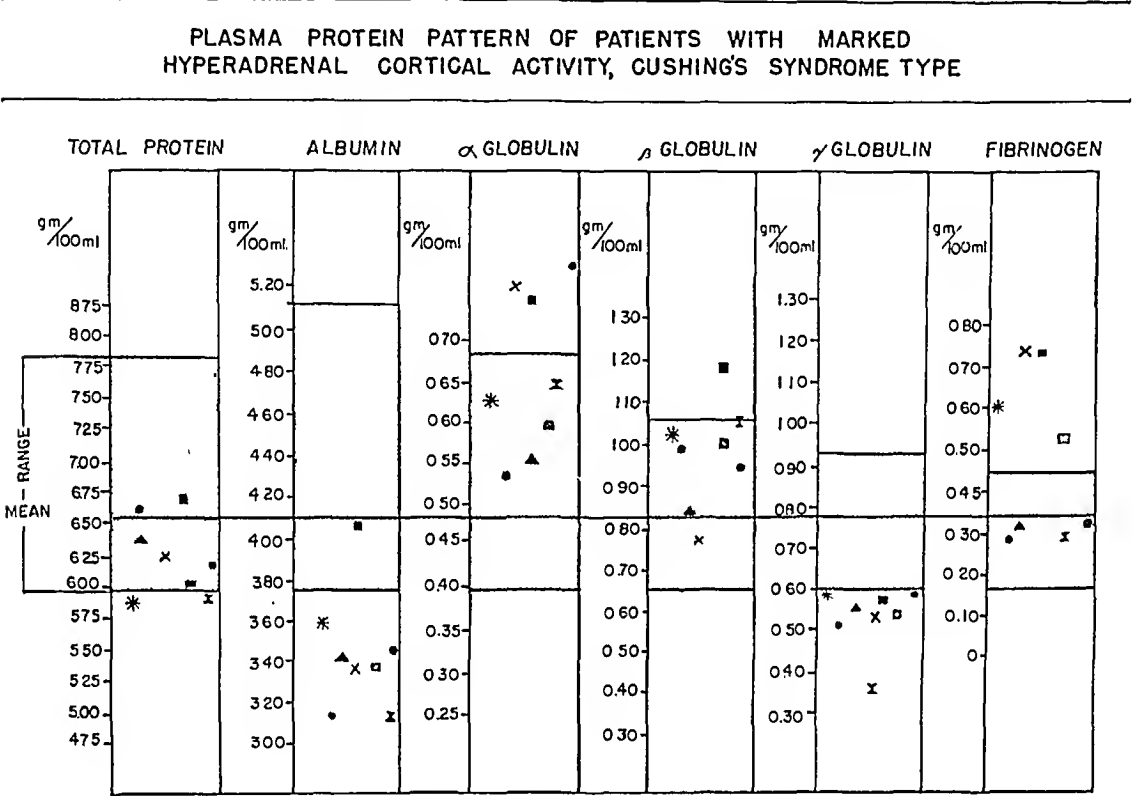


FIG. 1. Tiselius electrophoretic analysis of the plasma proteins of patients with Cushing's syndrome.

The plasma protein patterns of the 4 patients with acromegaly showed no consistent changes. Two of them who had moderately severe diabetes showed elevated  $\beta$ -globulin, similar to that found in patients with uncontrolled diabetes mellitus (4) without acromegaly.

DISCUSSION

The low plasma  $\gamma$ -globulin in these patients showing hyperadrenal cortical activity can be explained on the basis of the principles established by Dougherty, Chase and White (2) in experimental animals, namely, that administration of adrenal extract causes release of  $\gamma$ -globulin into the circulating plasma from lymphoid tissue. In the patient with hyper-

TABLE 1. CHANGES IN PLASMA PROTEIN PATTERN OF PATIENTS WITH CUSHING'S SYNDROME BEFORE, DURING AND AFTER THERAPY

Date	T.P.		A		α		β		γ		φ		Treatment and Clinical Conditions	Blood Pressure
	gm.	%	gm.	%	gm.	%	gm.	%	gm.	%	gm.	%		
Case Number 5														
5-11-43	5.49		3.11	56.6	0.51	0.3	1.00	18.3	0.52	0.4	0.32	5.0	Pretreatment	210/148
5-17-43 to 5-22-43			1200 r. total.										X-ray to pituitary	
6-22-43	6.57		3.90	59.4	0.60	10.0	1.25	10.1	0.30	5.0	0.37	5.0	X-ray to pituitary	185/140
6-21-43 to 6-26-43			1200 r. total.											
9-27-43													Hemiladrectomy of both adrenals	160/110
10-12-43													Some nausea	
12-6-43	6.27		3.51	56.5	0.48	7.6	1.14	18.3	0.63	10.1	0.48	7.6	Condition excellent	
10-7-46	7.20		4.31	40.7	0.43	5.0	1.25	17.2	0.80	11.9	0.38	5.3		
Case Number 6														
4-20-43	6.38		3.37	52.8	0.88	13.7	0.82	12.0	0.56	8.8	0.75	11.8	X-ray to pituitary	180/120
4-12-43 to 4-22-45			1200 r.		0.22-43 to 0.27-43			2460 r.					Partial removal both adrenals	145/100
9-14-43, 10-10-43													Looks and feels well, except for headaches	
11-21-41	6.01		3.65	50.7	0.68	11.4	0.95	15.8	0.70	12.6	0.57	9.5		
10-8-45	6.07		3.30	55.8	0.51	8.8	1.21	20.0	0.62	8.6	0.41	6.8	Looks normal except for obesity. Headaches have disappeared.	130/90
Case Number 7														
6-14-43	6.02		3.32	55.2	0.63	10.5	1.01	16.7	0.53	8.8	0.53	8.8		
6-4-44	6.07		3.28	51.1	0.47	7.7	1.01	16.6	0.88	14.5	0.43	7.1		
2-5-46	6.89		3.16	49.5	0.74	10.7	0.97	14.6	1.22	17.7	0.50	8.1		
Case Number 8														
1-5-38													Hemiladrectomy	
6-20-43	6.40		3.57	55.0	0.58	8.9	0.87	13.4	0.87	13.4	0.60	9.3	Excellent condition	130/92
			Normal (Average of 25 normals)											
	6.51		4.00	62.7	0.47	7.2	0.81	13.1	0.77	11.7	0.33	5.4		



adrenal cortical activity the initial change could be imagined to be a release of  $\gamma$ -globulin. At the time the studies reported here were made, the stimulation had been of such long duration that the  $\gamma$ -globulin reserves stored in the lymphoid tissue had been exhausted, and so the level in the circulating plasma could not be maintained. The blood lymphocyte count, in the 4 cases in which it was determined at the time of the protein study,

TABLE 2. PLASMA PROTEIN PATTERN (TISELIUS ELECTROPHORESIS) IN ACROMEGALY

Pa- tient Num- ber	T.P. gm. — 100 ml.	A		$\alpha$		$\beta$		$\gamma$		$\phi$	
		gm.		gm.		gm.		gm.		gm.	
		100 ml.	%	100 ml.	%	100 ml.	%	100 ml.	%	100 ml.	%
10	6.22	3.91	62.6	0.38	6.0	0.94	15.3	0.63	10.0	0.36	6.1
11	6.22	3.69	59.3	0.67	10.8	0.87	13.9	0.76	11.3	0.29	4.7
12	6.50	3.91	60.2	0.36	5.5	1.07	16.5	0.70	11.1	0.44	6.7
13	6.58	3.57	54.2	0.61	9.3	1.28	19.5	0.72	10.9	0.40	6.1

was very low, ranging from 615 to 2,000 per cu. mm. In the patients with hypoadrenal activity, either primary or of pituitary origin, the  $\gamma$ -globulin level was increased or at the extreme upper limits of normal. In these cases the  $\gamma$ -globulin reserves were adequate.

The marked increase in plasma protein level, especially in the  $\gamma$ -globulin fraction, following administration of testosterone (case 6) was interesting and agrees with the findings of Albright (1) which showed that administration of testosterone results in anabolism of protein. Protocols of the four patients who were observed over a period of years are appended.

*Patient No. 2.* The patient was first seen three years ago, then aged 24. He complained of arterial hypertension which he knew had existed for two years. He developed some degree of weakness, marked dryness of the skin, ease of bruising, striking roundness and redness of the face. Significant findings on examination included an appearance typical of Cushing's syndrome. Weight 146 pounds; height 63"; blood pressure 216/148. Blood count showed 5.5 million red cells; hemoglobin 13.5 Gm. Total blood volume was normal, showing 29 cc. of cells per kilogram. Water excretion test of Robinson, Power and Kepler showed an index of 21. Glucose tolerance was as follows:

	Fasting	$\frac{1}{2}$ hr.	1 hr.	2 hr.	3 hr.	4 hr.
Blood sugar	92	206	248	145	106	58
Urine sugar	0	—	0	Tr.	Ft. Tr.	0

17-Ketosteroids estimated on a pure ketonic fraction, totalled 6.9 mg. per 24 hours. X-ray revealed no enlargement of the sella turcica. There was moderate decalcification of

the bones of the skull; slight decalcification of the spine and pelvis and evidence of osteoarthritis of the hip and some calcification in the pelvic vessels. In September and October 1943, bilateral adrenal exploration was done. No tumor was found in either adrenal and a portion of each gland was removed. The portions of glands removed measured 1.3 by 0.7 cm. and 1.1 by 1.2 cm. respectively. No microscopic abnormality was found. In general his improvement has been slow but steady. Adrenal insufficiency was seriously entertained as a diagnosis during the latter part of 1943, in spite of the fact that his blood pressure had never attained normalcy. When last seen, in October 1946, his appearance was entirely normal. He was symptom free, working daily on the highway and on a farm. Blood pressure remained 180/130. There has been a gradual shortening of the right leg associated with the shortening of the neck of the femur and considerable absorption of the head and neck, associated with marked deposition of new bone. This change is apparently due to progressive aseptic necrosis plus osseous hypertrophy. Similar changes of milder degree are present in the opposite hip joint. The previous appearance of decalcification of the spine has almost completely disappeared.

*Patient No. 3.* Symptoms of this 22 year old woman were first noted  $4\frac{1}{2}$  years ago. These were chiefly increasing weakness, headaches and blurred vision. At the time of our first examination  $3\frac{1}{2}$  years ago, she presented the typical appearance of Cushing's syndrome with adiposity of the trunk, face and neck. Hypertrichosis, with a rather fine type of hair, involved the face, abdomen, shoulders and to some extent the breast area. The face was markedly round and red. She had been amenorrheic for three years. Typical purple striae were present. Blood pressure was 160/100. Red blood count was 5.29 million; hemoglobin 13.5 Gm. Spine by x-ray appeared to be osteoporotic. 17-ketosteroids were 2.9 and 3.8 mg. in 24 hours on two occasions. Basal metabolic rate varied from approximately -15% to -18%. Glucose tolerance was as follows:

	Fasting	$\frac{1}{2}$ hr.	1 hr.	2 hr.	3 hr.	4 hr.
Blood Sugar	111	193	292	246	238	122
Urine sugar	0	—	0	.33	.41	.40

X-ray therapy to the pituitary in doses of 2450 roentgen units delivered to each temple was given in April 1943 and since little or no improvement could be detected by September, 1943, bilateral partial adrenalectomy was performed. Considerable improvement followed this. Improvement, however, was very slow and for three years after operation, her weight was ten pounds greater than it was just prior to surgery, the weight being  $174\frac{1}{2}$  pounds; height 63". Spine had become much less osteoporotic. Blood pressure three years postoperatively was 145/100.

	Fasting	$\frac{1}{2}$ hr.	1 hr.	2 hr.	3 hr.	4 hr.
Blood sugar	96	139	161	161	157	89
Urine sugar	—	—	—	—	—	—

*Patient No. 6,* a white male, age 40 in 1946, has been under observation since January of 1937. His appearance is typical of that seen in the classical Cushing's syndrome. Outstanding features of the disease include a round red face with extremely plethoric appearance. He had persistent arterial hypertension, blood pressure usually being about 160/110. Typical purple striae were present. Red blood count has been as high as 6.02 million. X-rays of the spine and skull showed considerable demineralization as well as evidence of 15 rib fractures, all of which had been painless. X-ray therapy to the pituitary area was given in 1937 and in 1943 with little or no improvement. Therapy has included diet containing at least 100 Gm. protein per day and since 1943 protamine zinc insulin

15 to 20 units daily, as well as testosterone propionate 25 mg. two or three times weekly. In October of 1946, urinary corticoids measured by the method of Lowenstein and Corcoran were 23.2 mg. (Normal excretion is approximately 6 to 10 mg.) In November 1946, urinary 17-ketosteroids, measured on a pure ketonic fraction, were 23.9 mg. (Normal 7 to 14 mg.). In October of 1946 the glucose tolerance was as follows:

	Fasting	$\frac{1}{2}$ hr.	1 hr.	2 hr.	3 hr.	3 hr.
Blood sugar	112	216	272	316	208	157
Urine sugar	0	—	0	.42	.46	0

X-ray of the thorax and spine showed less demineralization than it had several years previously. Plasma potassium and sodium were normal. Blood urea was 45 mg.%, calcium 10.2, phosphorus 3.0 and cholesterol 191. The red blood count was 5.51 million; hemoglobin 13.5 Gm.

*Patient No. 7.* In 1937 this 26 year old white man was seen because of extensive epidermophytosis and onychomycosis. Two months previously and very suddenly severe pain occurred in the region of the lumbar spine, coming on while lifting. His appearance was typical of Cushing's syndrome and the history suggested that this appearance had developed over a period of the preceding 14 years. Glycosuria had been found on several occasions. His blood pressure was 188 systolic, 130 diastolic, height 67" and weight 165 pounds. The skull was demineralized, the vertebral column was markedly demineralized with a compression fracture of one vertebral body. Glucose tolerance test showed mild diabetes. Serum calcium, phosphorus, fasting blood sugar, cholesterol, urea were normal on repeated occasions. The blood count showed 5,550,000 red cells and 14.0 Gm. hemoglobin. Assays for androgens done by the capon comb growth method on four occasions were normal, varying between 33 and 65 international units in 24 hours. Perirenal air injection showed evidence suggestive of enlargement of the right adrenal. In January of 1938, exploration of the right adrenal gland showed it to contain a small adenoma and this gland was removed. In March of that year, he was also given 800 roentgen units of x-ray to each temple. His gradual improvement was interrupted by the development of a urinary calculus which was removed by ureteral meatotomy in August of 1939. In November 1939 his weight was 143 pounds, blood pressure 128/92. It was necessary for him to wear a back brace for about a year following surgery, after which this was discarded and he is apparently completely well. Plasma protein studies were done approximately four years after adrenal surgery.

### SUMMARY

Electrophoretic analysis of the plasma proteins of eight patients with hyperadrenal cortical activity (Cushing's syndrome type) showed low plasma albumin and  $\gamma$ -globulin level.

In two of the four cases studied following therapy, the plasma protein pattern tended to become normal. The plasma protein pattern of four patients with acromegaly was normal.

### ACKNOWLEDGMENT

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# THE CO-EXISTENCE OF HYPERTHYROIDISM AND PREPUBERAL EUNUCHOIDISM IN A MALE

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THE problem of the interrelationships between the thyroid gland and the gonads has been the subject of considerable study and controversy. Most of the reports concern themselves with the effect of thyroid activity on the gonads and only a rare reference has been made to the opposite relationship. These changes in gonadal function in the female have been reported frequently because menstrual disturbances lend themselves easily to clinical observation. Presumptive gonadal changes in the male have received much less attention and few reports have been published.

Not all observers believe that a direct relationship exists between these two endocrine organs. Van Dyke (13) concluded that thyroid activity probably has no significance in the regulation of gonadal function. Moore (9) stated that experimental data had not yet established the existence of any direct relationship between the thyroid and the testes although there was good evidence of the occurrence of indirect functional associations mediated probably by way of changes in oxygen metabolism. Salter (11) suggested 3 possible types of thyro-gonadal reciprocities:

- (1) a peripheral sensitization of tissues to the gonadal hormones by the thyroid;
- (2) effects produced by thyroid activity upon the gonadopituitary axis, or vice versa;
- (3) indirect effects of the thyroid upon the gonads via alterations produced in glands other than the pituitary, i.e. the adrenals.

Among the infrequent references to the effect of the gonads on the thyroid gland is that of Kinsell, Hertz and Reifenstein (4). They studied the effects of testosterone propionate and methyl testosterone in several thyrotoxic patients in whom weight loss and metabolic functions were prominent features. The testosterone propionate decreased creatine excretion, increased nitrogen retention, increased the patient's well being and weight without significantly affecting the basal metabolic rate. Methyl testosterone, on the other hand, aggravated the thyrotoxic state by virtue of its calorogenic action and its effect in increasing creatine excretion. From this study alone one could not conclude whether native androgenic substances are helpful or deleterious in thyrotoxic states because our knowledge of the metabolism of androgens in disorders of the thyroid gland is meager. It would be pertinent to know whether methylated androgens play any part in the intermediary metabolism of androgenic substances in

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patients with thyrotoxicosis. Since liver functions are frequently altered in thyrotoxicosis, it may be no surprise if, as a consequence, there were changes in steroid metabolism as well.

One would anticipate from Marine's theory of Graves' disease that the clinical administration of androgenic material would aggravate the thyrotoxic state. Marine (5) has long considered the hyperactivity of the thyroid



FIG. 1. Note the typical eunuchoidal measurements, hairlessness and extremely small external genitalia. Also note the stare.

gland in Graves' disease to be secondary to and dependent upon a primary anterior pituitary-gonad hormonal defect or anomaly which renders the individual's autonomic control over various visceral functions susceptible to injury by a great variety of non-specific influences. It is Marine's hypothesis that the gonadal disturbance consists of an imbalance in the androgen-estrogen ratio of the host favoring the preponderance of the androgenic function. In other words, there is presumed to be an absolute or a relative increase of androgenic over estrogenic substances. This androgen predominance stimulates the thyroid gland to produce more thyroid hormone which in turn tends to depress androgen production by its general depressing action on the anterior pituitary gland. The striking increase in

the incidence of Graves' disease at the menopause; the characteristic preservation, and at times hypertrophy, of the interstitial cells of the testes in Graves' disease in contrast to their involution in myxedema; the effect of successful iodine therapy in depressing the urinary excretion of androgenic material in true Graves' disease (6) plus the fact that no one had yet reported a case of Graves' disease in a male castrate (6) gave credence to Marine's hypothesis on the pathogenesis of hyperthyroidism. In the light



FIG. 2. Note enlargement of the thyroid gland.

of this theory the following report of a case of Graves' disease which developed in a typical prepuberal eunuchoid male is very interesting. In a search through the *Quarterly Cumulative Index Medicus* for the past 20 years, there is not a single reference to the co-existence of these two clinical syndromes.

#### CASE REPORT

*History:* The patient, a 28 year old white male, was admitted to the Beth Israel Hospital (#169228) on December 29, 1944 complaining of swelling of the neck of one year's duration, weakness and dizziness of one year's duration, and precordial and left elbow pain of 2 months' duration. About one year before admission the patient noted the

insidious onset of anterior swelling of the neck associated with an annoying, heavy and bounding pulsation. He also noted free and profuse perspiration, a feeling of warmth even in cold weather, palpitation, weakness, dizziness, tremor of the hands and increasing emotional instability. In the past year his weight had fallen from 170 pounds to 149½ pounds at the time of hospitalization. Precordial discomfort associated with some pain in the left elbow set in 2 months ago but these were not distressing enough to warrant medical attention at the time. For several months he had noted moderate dyspnea on moderate exertion but no orthopnea.

The patient's story was not completely reliable for he tended to change its context with every interrogation by members of the hospital staff. One of his private physicians



FIG. 3. Note the bilateral gynecomastia.

offered the information that the patient showed full evidences of hyperthyroidism with a tachycardia of 145 beats per minute when he was first consulted in 1943. On one following occasion the patient exhibited an episode of transient auricular fibrillation. His physician reported that the patient gave the history of having taken one thyroid tablet 3 times a day "for some time for the purpose of weight reduction." In the hospital, the patient denied having taken this drug steadily but admitted having ingested thyroid occasionally. The above mentioned physician also noted his bizarre general appearance which he interpreted as a Frohlich's Syndrome and treated him with a Parke Davis preparation of combined gonadotropic and pituitary hormone by injection twice weekly for 1½ months after which the patient ceased contact with the doctor. The only medications used to treat his hyperthyroid state were mild sedatives.

The past history included the congenital existence of undescended testes with an extremely small phallus and scrotum. He had never shaved. His body growth was rather rapid, especially his lower limbs. The voice was always high pitched and he admitted



finally that his libido was nil in spite of frequent falsification of this fact to several historians. The only treatment he ever received for this condition was that reported above by his physician. The treatment effected no change in his physical status. At the age of 10 he had a bout of rheumatic fever with polyarthritis and carditis for which he was treated at Bellevue Hospital at the time. There was no history of chorea, nor congestive heart failure and there had been no recurrences of rheumatic fever.

*Family History:* His mother had high blood pressure. Otherwise negative.

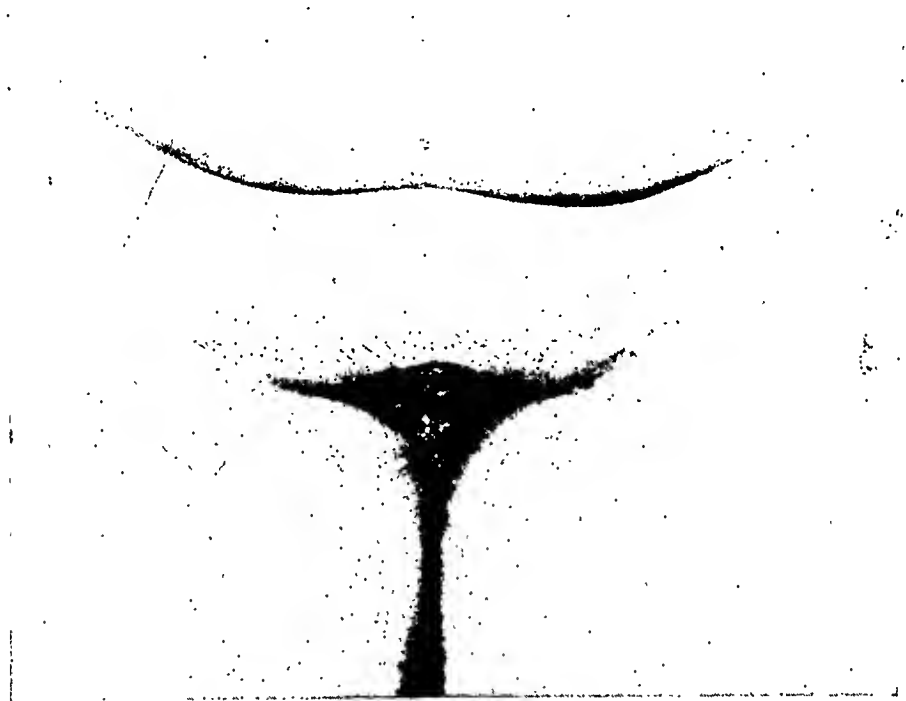


FIG. 4. Note the extremely small external genitalia and the few suprapubic hair follicles.

*Physical Examination:* The patient was a well nourished white male with a somewhat flushed, anxious expression. His body contour was feminine in appearance. The skin had a waxy smooth cast, was moist and hairless except for a few suprapubic hair follicles. The voice was high pitched. The eyes exhibited a distinct stare, slight bilateral exophthalmus, slightly widened palpebral fissures and a lid lag. The neck revealed a diffusely enlarged, firm, bulging thyroid gland over which could be heard a systolic bruit. The lungs were clear. The point of maximum cardiac impulse was visible and palpable in the sixth intercostal space outside the mid-clavicular line. The heart sounds were of good quality. The rate was regular and rapid at 130 beats per minute. A2 was louder than P2. Double apical and basal murmurs were audible. The blood pressure ranged between 110-130 mm. systolic and 60-0 mm. diastolic. A bilateral gynecomastia of moderate degree was present. The abdomen was negative. The extremities showed the measurements typical of prepuberal eunuchoidism. The distance from the symphysis pubis to the feet was 36 inches compared to the measurement of 32½ inches from the top of the head to the symphysis pubis. The palms were moist and warm. The deep reflexes were exaggerated and there was a fine tremor of the outstretched hand. A slight to moderate degree of thoracic kyphosis was evident. The phallus was extremely small. The testes were

undescended and could not be palpated in the inguinal canals. The scrotum and prostate gland were also extremely small.

*Laboratory Data:* Urine was negative. The red blood cell count was 4.41 million per cubic millimeter and the hemoglobin was 11 grams. The white blood cell count was 7,700 with 64 polymorphonuclears, 1 basophil, 28 lymphocytes and 7 monocytes. The Wassermann, Kline and Kahn tests were negative. The non-protein-nitrogen level of the blood was 33 milligrams per cent. An oral glucose tolerance test revealed a fasting blood sugar level of 96 milligrams per cent followed by levels of 122, 96, 80 and 78 milligrams per cent at hourly intervals after the ingestion of a single dose of 100 grams of glucose. The serum cholesterol before any treatment was instituted ranged between 120 and 130 milligrams per cent on 3 occasions. The creatine excretion in urine was 255 milligrams in 24 hours. The creatinine excretion in urine was 1,253 milligrams in 24 hours. The total serum protein was 4.18 grams per cent with serum albumen of 2.49 grams per cent and serum globulin of 1.69 grams per cent. The insulin tolerance test showed a fasting blood sugar level of 98 milligrams per cent with one-half hourly readings after 7 units of insulin intramuscularly (0.05 unit per pound of body weight) of 96, 63, 66 and 86 milligrams per cent. The visual fields were normal. The electrocardiogram was normal. The x-ray of the skull was normal. The sella turcica was within normal limits. The 17-ketosteroid excretion in the urine was reported\* as alpha fraction 2.7 milligrams in 24 hours (normal 8-15 mg.) and beta fraction 0.07 milligram in 24 hours (normal 0.3-4 mg.). On January 6, 1945, the blood total iodine content\* was 8.4 micrograms per cent (normal 2-4).

*Course:* On January 29, 1945, treatment of the patient's hyperthyroidism was started with the initial daily dose of 1.2 grams of thiouracil and 150 mg. of pyridoxine. Clinical improvement was rapid and dramatic. As his condition improved, the dosage of thiouracil was decreased to 0.2 gram daily which was the dosage at the time of his discharge from the hospital on February 20, 1946. The fall in the basal metabolic rate paralleled the disappearance of the signs and symptoms of thyrotoxicosis. The weekly BMR determinations before institution of thiouracil were plus 35, plus 36, plus 42 and, following treatment, plus 44, plus 31, plus 9 and minus 3 per cent at the time of discharge from the hospital. His weight which diminished during hospitalization from 149½ pounds to 144½ pounds rose to 147 pounds after thiouracil treatment. There was no change in the total leucocyte or differential blood count while in the hospital. He was referred for follow-up to the Metabolic Clinic of the hospital. There his co-operation was very poor, his attendance erratic and his behavior delinquent. In April 1945, while under thiouracil therapy he developed a moderate granulopenia which soon reversed itself on cessation of therapy. He refused to re-enter the hospital for observation and change in the form of therapy. Nothing was heard of him until he returned to the medical clinic on September 24, 1945 and again on October 15, 1945, showing auricular fibrillation and marked thyrotoxicosis. His blood picture was within normal limits. He was again urged to enter the hospital but again refused. No further information about his status was obtained until it was learned that he died at home in March 1946. Communications with his family physician revealed that his course was rapidly downhill. The signs of thyrotoxicosis became more marked and auricular fibrillation became chronic in September 1945. His weight declined rapidly until he developed marked congestive heart failure which was unaffected by digitalis and dehydration measures. Necropsy examination was not performed.

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\* The 17-ketosteroid and blood iodine determinations were performed by Dr. E. J. Baumann of Montefiore Hospital, New York City.

## DISCUSSION

From both a clinical and laboratory viewpoint, it is evident that this individual presented the co-existence of two endocrinopathic syndromes, namely eunuchoidism and hyperthyroidism. This combination of glandular disturbances has not been reported in the literature heretofore. In several studies of eunuchoid males it was found that the basal metabolic rates were always below normal or normal (3, 8). The usual range of the basal metabolic rates in eunuchoid males was  $-15$  to  $-20$  per cent with an occasional low level of  $-30$  per cent (3, 8). The mechanism for this hypometabolic state in eunuchoidism is not fully understood. It may be due to the fact that with the marked decrease in androgenic material the sensitization of the peripheral tissues to thyroid hormone is diminished. This would be the counterpart of the increased sensitivity to thyroid extract exhibited by eunuchoids after treatment with testosterone propionate as described by Eidelsberg and Ornstein (2). Other possible explanations for this hypometabolic state are (a) the so-called crowding-out of the thyrotropic factor of the anterior pituitary by the increased production of gonadotropic substance when the eunuchoidism is primarily testicular in origin and (b) the presence of panhypopituitarism when the eunuchoidism is of pituitary origin.

In the light of our present knowledge, it is difficult to understand the pathogenesis of the association of hyperthyroidism and prepuberal eunuchoidism as presented above. If, as Aron and Benoit (1) believed, castration increased the production of the thyrotropic as well as the gonadotropic hormones of the anterior pituitary gland with consequent increased activity of the thyroid gland, one would expect the co-existence of Graves' disease and castration with greater frequency than actually exists. Moore (10), on the other hand, stated that no severe changes appear to develop in the thyroid gland after castration. McCullagh and Walsh (7) could demonstrate no change in the size of the thyroid gland in rats 85 days after castration.

The possibility exists that the patient we have just described had ingested exogenous thyroid substance to the point of permanent hyperthyroidism. Such an occurrence is, however, rare, particularly in syndromes characterized by a hypometabolic state such as exists in eunuchoids. Schoeller and Gehrke (12) reported that male castrate mice on a similar dose of thyroxine (0.5 mg.) showed a 50 per cent less rise in metabolism than normal mice. On the other hand, as stated above, Eidelsberg and Ornstein (2) found that there was an increased sensitivity to thyroid in eunuchoids who had been treated with testosterone propionate and definite hypermetabolic rates in eunuchoids without Graves' disease were reported

by McCullagh (8) after prolonged treatment with large doses of methyl testosterone. The patient reported here had never been treated with testosterone products for his eunuchoidism. The relationship of the superimposed Graves' disease to the underlying and antecedent eunuchoidism remains therefore a puzzle defying explanation with our present limited knowledge regarding the thyro-gonadal axis. It is greatly regretted that necropsy examination was not performed for it may have contributed to a clarification of the clinical pathological problem. One wonders what the adrenal cortex or perhaps the hypothalamus showed in this case and what relationship they may have had to the thyro-gonadal axis.

### SUMMARY

The very unusual co-existence of hyperthyroidism and eunuchoidism in a male is reported. The relationship between the two clinical syndromes is unknown.

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# USE OF MASSIVE DOSES OF VITAMIN A IN THE TREATMENT OF HYPERTHYROIDISM

## A PRELIMINARY REPORT

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INVESTIGATIVE work during the last decade and a half has established the close interrelationships existing between hormones and vitamins in their effects on the same organs of the body (19). Of considerable interest is the relationship between thyroxine and vitamin A, concerning which an extensive literature has developed abroad but strangely enough comparatively little in this country. The antagonism between the thyroid hormone and vitamin A was first suggested a half century ago (39) and confirmed between 1930 and 1939 by extensive experimental work in animals.

For the present report the effects of massive doses of vitamin A (200,000 to 400,000 I.U. daily) in hyperthyroidism were tested in two patients. One was a woman with menopausal exophthalmic goiter of moderate severity complicated by essential hypertension; the second, a rather severely toxic hyperthyroid woman. Routine studies such as urinalysis, blood count, blood sugar and electrocardiogram were performed in both patients; the metabolic rate and blood cholesterol were determined as in Table 1; the weight and pulse rate were checked semi-weekly, as was the blood pressure, in *case 1*. For ease of administration the vitamin A was dispensed in divided dosage in capsules containing 50,000 I.U. each.<sup>1</sup>

## CASE REPORTS

*Case 1.* Diagnosis: Menopausal exophthalmic goiter with essential hypertension.

*Mrs. R. L.*, a white woman aged forty-six, an inspector of clothes, had been in good health until 1943, a year before she became our patient, when asymptomatic hypertension was discovered. The menses had been irregular for two years and during this time she suffered from mild vasomotor phenomena (flashes, sweats and chilliness). She had been married nineteen years and had one child alive and well. Appendectomy was performed in 1917.

Five weeks before she came to the hospital she had noticed marked progressive irritability and nervousness, tremors of the hands and increased perspiration. Her appetite was good but her weight had fallen during this period from 110 pounds to 105 pounds. Palpitation had been present for three weeks. During the last week she had suffered from

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insomnia. A sensation of substernal pressure developed after dinner. She had been forced to leave her work at the onset of her symptoms.

**Physical Examination:** The patient was short and slender weighing only 105 pounds. Her skin was flushed and moist. Slight exophthalmos was present with definite lid lag. The thyroid gland was slightly diffusely enlarged, especially the right lobe, and was smooth. The pulse-rate ranged between 100 and 120 per minute. The heart sounds were booming. The heart and lungs were normal. The abdomen revealed a right lower quadrant scar. A fine tremor of the hands was present. The reflexes were normal, as was sensation.

**Laboratory Data:** The basal metabolic rate was +44 and the blood cholesterol 160 mg. per cent. Urinalysis was normal. The blood count revealed hemoglobin 13.0 Gm.,

TABLE 1. CORRELATION OF BASAL METABOLIC RATE AND BLOOD CHOLESTEROL WITH TREATMENT BY VITAMIN A AND LUGOL'S SOLUTION

Duration Treatment (weeks)	Basal Metabolic Rate	Blood Cholesterol (mg. %)	Vitamin A (i.u. daily)	Lugol's Solution (minims daily)
<i>Case #1</i> control	+44	160	300,000	0
<i>R.L.</i> 4½	+12	300	300,000	0
11	+ 6	260	300,000	0
14½	—	—	0	0
17½	0	330	0	0
19½	—	—	200,000	0
24½	- 1	296	200,000	0
<i>Case #2</i> control	+53	175	400,000	0
<i>L.Q.</i> 3	-11	190	400,000	0
5½	+ 4	199	400,000	0
19	+ 7	269	400,000	0
32	- 2	266	400,000	0
47	—	—	300,000	10
51	+ 6	223	300,000	10

red blood cells 4,300,000/e.mm., white blood cells 6,200/e. mm., with neutrophils 68 per cent, lymphocytes 20 per cent, and monocytes 12 per cent. The blood sugar was 105 mg. per cent. The electrocardiogram was normal. X-ray showed no defects in the heart or lungs.

**Progress:** The administration of 300,000 i.u. daily of vitamin A was begun June 30, 1944 (Table 1). After one week the pulse-rate was 108/minute and severe headache was present, but the feeling of nervous tension had disappeared. Insomnia was still present. After three weeks' treatment the pulse rate had fallen to 88/minute, the weight had increased three pounds and all symptoms, except mild flashes and substernal pressure after dinner, had disappeared. A week later the patient was totally asymptomatic, the basal metabolic rate had fallen to +12, the blood cholesterol had risen sharply to 300 mg. per cent and the weight was stationary. Steady improvement continued thereafter

with further reduction in pulse rate to 66/minute, progressive increase of weight to 120 pounds, continued fall of basal metabolic rate, maintained increase of blood cholesterol and a progressive diminution in the size of the thyroid gland until the fourteenth week of treatment when vitamin A therapy was discontinued. At this time the patient resumed her work. The thyroid gland was now entirely normal to palpation.

Vitamin A administration, 200,000 i.u. daily, was resumed after a lapse of five weeks for the treatment of the persistent hypertension and was discontinued ten weeks later. The basal metabolic rate continued to fall, as did the pulse rate, and the blood cholesterol remained at approximately the same level. The blood pressure fell from an average of 188/119 to 148/101. The patient has been under continuous observation for thirty-six months since treatment was first begun and has remained entirely normal except for a moderate hypertension. During the last thirty months she has not received vitamin A.

#### *Case 2. Diagnosis: Hyperthyroidism*

*Mrs. L. Q.*, a white thirty year old housewife had, four years prior to her present illness, exhibited irritability for two months, tachycardia, a slight weight loss, marked tremors of the hands and a marked, diffuse, soft enlargement of the right lobe of the thyroid gland. The basal metabolic rate was +22 and the resting pulse rate 88/minute. She had been treated four weeks with Lugol's solution, minims 30 daily, with resultant quiescence of symptoms. Her past medical history was otherwise negative.

At the time of her admission to the hospital, June 16, 1945, her symptoms were extreme nervousness, palpitation and a weight loss of eight pounds in four weeks despite a ravenous appetite. Her symptoms seemed to date from the shock of her husband's induction into military service.

**Physical Examination:** The patient was of medium height and slender. The temperature was 99° F., the blood pressure 104/70, the weight 101 pounds, and the pulse rate 108 to 140/minute. The entire thyroid gland was diffusely moderately enlarged and firm. The right lobe felt slightly nodular. The skin was moist. No ocular signs were present. She exhibited fine tremors of the hands. Examination was otherwise essentially negative.

**Laboratory Data:** The metabolic rate was +53 and the blood cholesterol 175 mg. per cent. Urinalysis was normal. The blood count revealed hemoglobin 12.8 Gm., red blood cells 4,400,000/c.mm. and white blood cells 6,500/c.mm. with normal differential. The blood sugar was 92 mg. per cent. The electrocardiogram was normal. X-ray showed a normal chest.

**Progress:** Treatment was begun with 400,000 i.u. of vitamin A daily. The basal metabolic rate (Table 1) plummeted to -11 after three weeks of treatment and the blood cholesterol increased slightly to 190 mg. per cent. The pulse rate fell to 80, and the tremors of the hands became less marked. The goiter became definitely smaller. She felt improved, with a marked decrease of nervousness and irritability. After five and a half weeks of treatment the basal metabolic rate was +4, and the blood cholesterol 199 mg. per cent. The pulse rate was 116, an elevation apparently due to premenstrual tension (as will be discussed below). After seven weeks' treatment the goiter had regressed greatly in size, was still diffuse but no longer nodular. Thereafter her course continued uphill with slow but progressive improvement in pulse rate and weight, as well as a continuance of normal basal metabolic rate and further increase in blood cholesterol values. The smoothness of her course thereafter was interrupted only by symptoms of premenstrual tension.

After forty-two weeks of treatment the pulse rate showed persistent elevation (86 to 96/minute) from its preceding lower levels, but the patient felt well, maintained her improvement and showed no signs or symptoms of toxicity. However, in an attempt to forestall any possibility of a recrudescence of toxicity, Lugol's solution (10 minims daily) was added to the vitamin A therapy at the forty-seventh week. The iodine produced no additional effect on the basal metabolic rate (+6) or the blood cholesterol (223 mg. per cent) which was determined four weeks later, but did decrease the pulse rate to 78/minute, at which level it has remained. All medication was now discontinued. The patient has remained perfectly well during the twelve months she has been under observation since vitamin A therapy was discontinued. Her weight reached 111 pounds (a gain of 10 pounds). The thyroid gland is barely palpable, and the patient appears to be entirely cured of her hyperthyroidism.

### Hyperthyroidism

Proof of the antagonism between vitamin A and the thyroid hormone rests on an extensive laboratory and clinical background. The use of thyroxine prevents the production of hypervitaminosis A in animals (20). Hyperthyroidism in rats can be counteracted by a diet rich in vitamin A and B (2), or by cod-liver oil (60). Soskin and Mirsky (50) cured a very toxic hyperthyroid patient by use of a high fat diet (230 Gm. of fat daily). Vitamin A counteracts the rise in basal metabolism produced by large doses of thyroxine and ameliorates the toxicity (3, 31, 38, 57).

Abroad, vitamin A has been used successfully in the treatment of human hyperthyroidism. In Germany, Wendt (57, 58) achieved excellent results in six patients with moderately severe to severe hyperthyroidism by the use of 150,000 to 240,000 i.u. of vitamin A daily over periods of time ranging up to twelve weeks. Falta (21) reported a cure in a patient with markedly toxic hyperthyroidism. Dietrich (13) obtained excellent results in five of six patients treated for several weeks; the concurrent use of iodine seemed to enhance the therapeutic results obtained. Goth (26) reported good results in six of seven cases treated for several weeks with 100,000 to 200,000 units daily. Tislowitz (52) cured one patient by the administration of three to four glasses of carrot juice daily.

In France vitamin A therapy has been utilized only on a rather small scale. Sendrail (48) used small doses of vitamin A (2400 i.u. daily; 25,000 i.u. daily in one case only) combined with Lugol's solution and secured promising results in the majority of patients. Azerad (5, 6) used 120,000 i.u. daily in a large number of cases and obtained complete success in the majority, but only after prolonged treatment. In a second group which had been treated successfully with iodine or vitamin A alone the symptoms recurred after cessation of treatment; when vitamin A was combined with iodine the results were excellent. In a third, rather small, group the combination of vitamin A and iodine proved a complete failure. Rymer (43)



secured much more rapid improvement with vitamin A plus iodine than with the use of iodine alone. In general, the Germans used a much higher dosage of vitamin A comparatively than the French did.

Elsewhere abroad Mariante (33), employing 180,000 to 240,000 i.u. of vitamin A daily, obtained good results in eight of fourteen cases. Casassa and Pescarmona (10) secured relief in hyperthyroidism with moderate dosage of vitamin A.

The striking feature in the present series is the rapid fall of the basal metabolic rate in *cases 1* and *2*, being extremely precipitous in the latter case and approaching that ordinarily obtained with the use of iodine. The successful cases reported in the literature showed that the basal metabolic rate usually required at least seven weeks to be restored to normal. Many authors, especially the French (6), emphasize that several months may be required for the basal metabolic rate to fall to normal levels. In all probability the more rapid results obtained in this series may be ascribed to the relatively much larger dosage employed.

Keeping pace with the fall in basal metabolic rate was the decrease in pulse rate which fell to normal levels within three weeks in both cases. As striking was the amelioration of symptoms, being definitely pronounced within one and a half to two weeks in both cases. The weight was much slower in attaining its maximum and varied with each case insofar as rate of increase was concerned. In *case 1* the weight began to climb only at the end of seven weeks' treatment. In *case 2* the weight showed very little increase until thirty-two weeks had elapsed when it began to increase sharply coincident with a sudden pronounced fall in the pulse rate. We must conclude that subclinical toxicity was present in this patient until this point.

There is an increased need for vitamin A in hyperfunction of the thyroid gland (47), the blood content of vitamin A in human beings suffering from hyperthyroidism falling even to zero despite adequate intake (63). It is noteworthy that there exists a certain accord between the fall of blood vitamin A level and the augmentation of the basal metabolic rate. Treatment of the hyperthyroid patient with vitamin A produces an elevation of vitamin A in the blood serum that is indicative of improvement (4) and comparable with the rise of blood cholesterol (30). The fall of vitamin A in the blood serum has been advanced as a diagnostic aid in the determination of hyperthyroidism (51).

Within three to four weeks after treatment was begun in the present cases the goiter began to decrease in size. This is similar to the experience of Fasold (22). Regression then continued at an accelerated rate, with complete disappearance of the goiter in as little as fourteen weeks in one case.

## Blood Cholesterol

The blood cholesterol value is low in toxic thyroid states (27, 28). The fall in cholesterol value in hyperthyroidism has been attributed to the chronic, toxic, interlobar, parenchymatous hepatitis which is present in 50 to 75 per cent of hyperthyroid patients (7). Wendt (59) showed that vitamin A produces a hypercholesterinemia, with some patients showing a rise to 250 mg. per cent or higher. An attempt has been made to show that vitamin A is the catalyst in the rise of the serum lipoids which follows vitamin A administration (59). Dzialoszynski (15) has pointed out that vitamin A occurs in human blood in some sort of association with a plasma protein (probably serum albumin), cholesterol and lipoids.

The blood cholesterol in the present series showed the same inverse relationship with the basal metabolic rate as follows the use of iodine in hyperthyroidism. The increase was very striking in *case 1*, showing a steady rise even two weeks after the use of vitamin A had been discontinued. What proportion of the rise in cholesterol was due to the fall in the basal metabolic rate and what proportion to the ability of vitamin A to produce hypercholesterinemia is not clear (59).

## Vitamin A and Iodine

The synergism of iodine and vitamin A in the treatment of hyperthyroidism has been mentioned. In *case 2* the addition of iodine seemed to hasten the reduction of the pulse rate. During thyrotoxicosis the glycogen and vitamin A reserves of the liver are depleted (18, 56). A point of interest made by Schneider is that the use of vitamin A combats the fall in glycogen content of the liver so adequately that iodine can be dispensed with (44).

## Effects of Vitamin A on the Sexual Mechanism

Vitamin A produces significant effects on the sexual mechanism, although our knowledge of the subject is rather meager (22, 34, 62). There is evidence to indicate the specific important role of vitamin A in the hypophyseal-gonadal interaction (54).

In *case 1* the menses were irregular before treatment was begun and showed no change under treatment. In menopausal hyperthyroidism we are concerned with excess of thyrotropic hormone (49). The probable mechanism whereby vitamin A was of value in this case may be by virtue of diminution of the thyrotropic hormone through direct action of vitamin A, or possibly through inhibition of the gonadotropic hormone which normally stimulates production of the thyrotropic hormone. *Case 2* had had regular menses every thirty-five days, lasting three to four days.

Under vitamin A treatment the menses tended to come closer together, ranging from twenty-six to thirty-four days. There was little, if any change in the flow. An interesting feature was that at the forty-seventh week of treatment, during the hot July weather, she developed numerous flashes and a sensation of chilliness of a mild character that became very slight in the course of four weeks and disappeared completely during the next week. These symptoms may be ascribed to a diminution in ovarian function.

Case 2 also displayed some of the group of symptoms now well known under the term "pre-menstrual tension" (61): increased subjective nervousness, restlessness, depression and crying spells. The symptoms began three days before, and ceased promptly with the onset of the menses. They became progressively less marked and finally disappeared completely after thirty-two weeks of treatment with vitamin A. The mechanism is open to question, possibly involving a decreased formation of circulating estrogen.

### Action of vitamin A

The actual mechanism by which vitamin A is effective in the treatment of hyperthyroidism is still unknown. Several theories have been advanced. Spectroscopic methods have demonstrated that thyroxine and carotene *in vitro* are bound together, thereby inactivating the thyroxine (18, 24). There is no proof that such a direct chemical inactivation takes place *in vivo*. Schneider thought that the action was peripheral, protecting the liver against the action of thyroxine tending to lower the reserves of glycogen in the liver (45). Abelin felt that the antagonism between thyroxine and vitamin A was explainable by their opposing effects on the lipid metabolism of cells and the glycogen metabolism of muscle (3). Fasold and Peters, as well as Abelin, postulated a direct antagonism between vitamin A and thyroxine (1, 20). Others concluded that vitamin A has a direct inhibitory action on the complex hypophyseal thyrotropic hormone, preventing the liberation of thyroxine (17, 23). Belasco and Murlin concluded that possibly vitamin A, by virtue of its double bonds, is able to take up the available iodine in the body tissues and, either by acting on the thyroid gland or the anterior pituitary gland directly, affect the storage of colloid with consequently lowered oxygen uptake of the thyroid gland (8). McCarrison's conclusion that the thyroid gland is unable to deal with iodine in the normal manner in the absence of a sufficiency of vitamin A, with goiter resulting, is amply supported (12, 32, 35, 42).

### Toxicity of Vitamin A

The effects of hypervitaminosis A in animals are well established. Trophic changes occur in the skin, bones and bone marrow. The skin

lesions, which appear first, consist of coarsening of the hair, ring crusts on the tail and inflammatory changes in the conjunctiva and nose (37, 62). The skeletal lesions consist of an atrophy of the long bones which become very thin and brittle, often resulting in spontaneous fractures (9, 11, 16). The fractures are due to a negative calcium balance with drastic decalcification of the long bones and vertebral column (9, 11, 41). There is a marked tendency to hemorrhage (36). Other pathology reported in animals includes focal liver necrosis, toxic degeneration of striated muscles and testis and a glomerulo-nephritis with calcification which is claimed as the usual cause of death (14).

In human beings the literature concerning the toxicity of hypervitaminosis A is rather meager. As long ago as 1596 it was recognized that polar bear and seal (*Phocabarbata*) liver could produce illness in man (loss of skin from head to foot) (40). Other expeditions proved this to be due to the extremely large value of vitamin A in polar bear and seal liver and found that toxicity develops in man with an ingestion of 7,500,000 I.U. of vitamin A daily (41). Schneider took a dose of 900,000 I.U. of vitamin A with the production of only light perspiration and a feeling of anxiety (46). Lehman and Rapaport used a single dose of 2,000,000 I.U. as a test for visual deficiency in children. They also prescribed 100,000 to 200,000 I.U. daily for months without discernible bad effects (29).

Note should be made of the extremely high dosage at which toxic effects begin in man. Until now the Germans used the highest dosage (up to 250,000 I.U. daily) without toxic effects; in the two cases reported here dosage as high as 400,000 I.U. daily over a long period of time was employed without ill effects. This lack of toxicity is to be expected when we compare even the massive doses used here with the relatively enormous doses required to produce illness in man (a safety margin of approximately 20 to 1). This is further attested by the lack of toxicity in a group of 100 hypertensive patients treated with similar dosage over periods of eighteen months.<sup>2</sup> The only other possibility of harmful effects rests with the occurrence of unusual sensitivity to vitamin A (25).

Normally no vitamin A is found in the stool (55) and never in the urine except under certain pathological conditions (53). No vitamin A is excreted in the feces under an ingestion of 25,000 I.U. daily; about 3 per cent to 4 per cent is excreted on a dosage of 76,000 I.U. daily, and an increasingly higher percentage as the intake is increased (55). Apparently the excess of vitamin A is rapidly destroyed in the body. From the data presented here it is permissible to conclude that even massive doses of vitamin A may be safely used in human beings over periods of many months.

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<sup>2</sup> Work to be published by author.

## SUMMARY AND CONCLUSIONS

1. Extensive experimental work in animals and clinical work in human beings in Germany, France, England and elsewhere abroad during the last seventeen years has proved conclusively the antagonism between vitamin A and the thyroid hormone, as well as the great value of vitamin A in the treatment of human hyperthyroidism.

2. Vitamin A was used in two cases of hyperthyroidism in massive doses (200,000 to 400,000 i.u. daily) for periods up to fifty-one weeks.

One case of menopausal exophthalmic goiter with hypertension, treated fourteen weeks, showed complete cure of the hyperthyroidism; continuous observation for thirty months subsequently has revealed no recrudescence. One case of hyperthyroidism with severe toxicity, treated fifty-one weeks, resulted in complete cure; continuous observation for twelve months subsequently has revealed no recrudescence.

3. The thyroid enlargement regressed within three weeks and disappeared within fourteen weeks. The slight exophthalmos which was present in one case disappeared completely under treatment.

4. Vitamin A acts very much like iodine in producing a rapid decrease in the basal metabolic rate, increase in the blood cholesterol and marked amelioration of symptoms, but a slower decrease of pulse rate and increase in weight.

5. The addition of iodine to vitamin A therapy may enhance the effectiveness of vitamin A.

6. Vitamin A produces significant effects on the sexual mechanism, especially ovarian function. Vitamin A produced a cure of the symptoms of pre-menstrual tension in one patient.

7. A marked maintained fall in blood pressure was obtained in the patient with menopausal exophthalmic goiter and hypertension.

8. The mechanism by which vitamin A is effective in the treatment of hyperthyroidism is still a matter for speculation.

9. Vitamin A is innocuous, even in doses of 400,000 i.u. daily over periods ranging up to fifty-one weeks.

10. Vitamin A is a promising therapeutic agent in the treatment of hyperthyroidism, both for cure and for alleviation of the toxicity.

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# GYNECOMASTIA

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**G**YNECOMASTIA was first described by Basedow in 1848 (26). Since that time isolated reports of this condition have appeared in the literature. In 1938 Glass and Bergman (12) demonstrated that the ratio of the urinary androgens to the urinary estrogens in patients with gynecomastia was lower than normal, approaching the ratio found in females.

In 1942, Klinefelter, Reifenstein and Albright (20) presented a syndrome characterized by gynecomastia, aspermatogenesis, aleydigism and increased urinary excretion of the follicle stimulating hormone. They discussed the possible endocrine relationship in its etiology. Investigating similar cases in 1945, Heller and Nelson (15) showed that gynecomastia was not a constant sign of the syndrome and suggested a different etiology for the enlarged gland.

During World War II gynecomastia assumed the aspect of an occupational disability in the Armed Services because of the mechanical irritation of the enlarged gland and the psychic embarrassment resulting from communal living conditions.

The term, gynecomastia, refers to a unilateral or bilateral enlargement of the male breast which occurs as a result of hyperplasia of duct epithelium and periductal stroma. The term is not used in reference to those enlargements of the male breast occurring as a result of neoplasm, infections, cysts, and increased adipose tissue. The last named condition is referred to as pseudogynecomastia (11).

## INCIDENCE

Webster (28) reported an incidence of 6.96 to 8.69 cases of gynecomastia per 100,000 Navy personnel during the years 1939 to 1942 inclusive.

A direct communication to us from the Army Service Forces, Surgeon General's Office, Washington, D. C., states: "The admission rate for gynecomastia in the United States Army is about 13 per hundred thousand per year."

In 1943, while prisoners of war, Major Robert Lewis (30) of the United States Army Air Forces and Colonel W. D. North of the United States Army Medical Corps observed among approximately 3,200 American prisoners of war who had spent nearly one and one-half years in Japanese

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prison camps, numerous men with a single, quarter-sized, smooth, sub-areolar nodule in the breast. These lesions were unilateral or bilateral. A survey of all personnel in one day by Major Lewis revealed an incidence slightly over four per cent. Many of these nodules disappeared spontaneously. Microscopic studies were not possible at the time.

The pathology laboratory's records of the United States Army medical installation, Brooke General Hospital (31), show that 72 cases, called gynecomastia by the pathologist, were operated on in 1945 and ten cases during the first three months of 1946. Grossly, many of the cases were reported as a nodule in the breast, while an equal number were listed as a diffuse enlargement. During 1945 the records of the surgical section (32) at Brooke General Hospital listed a total of 7,281 operative procedures being performed.

### ETIOLOGY

A multiplicity of factors have been cited as the cause of gynecomastia. The etiology is frequently obscure. Inasmuch as normal breast hypertrophy in the female has been demonstrated to be the result of the action of the ovarian hormones, especially estrin (2, 27), it is natural to think of estrogenic hormones when considering mammary gland enlargement in the male. However, as demonstrated by Corner (2), pituitary injections alone have produced mammary gland hypertrophy in ovariectomized virgin rabbits.

The general consensus revealed by a review of the literature is that gynecomastia is a result of an altered endocrine status. Quantitative estrogen and androgen tests made on the urine of a number of patients with gynecomastia showed the A:E (androgen:estrogen) ratio to be altered substantially. Glass and Bergman (12) found with their methods an average urinary A:E ratio of 15.3 for normal males and 2.3 for normal females; the A:E ratio in their two cases of gynecomastia was consistently around 5.0.

Menville (22) feels that mechanical stimulation or irritation may play a very important role in male breast hypertrophy; yet he was able to obtain a history of actual trauma in only 14.5 per cent of his cases. Other investigators have recorded a similar lack of evidence for the mechanical irritation theory. It is difficult to determine whether the condition is a result of trauma or made noticeable by trauma. The high incidence in the army is felt by some to be the result of training or combat. The prolonged wearing of straps and packs is considered the greatest contributory factor.

Trauma may be an associated factor in individuals undergoing hormonal changes, but it cannot be considered the direct cause. It is difficult to explain a unilateral gynecomastia on the basis of trauma caused by

straps or packs which produce equal pressure on both sides of the chest.

The breast lesions reported by Lewis and North (30) among American prisoners of war are of interest in view of the obvious avitaminoses which were present at the time. The avitaminoses could have a direct bearing or could have acted indirectly by disturbing the endocrine production in the individual.

An hereditary factor is discredited in most instances but may be responsible for the condition known as pseudo-gynecomastia or enlargement of the breast area by adipose tissue without increase in actual glandular tissue. (28).

On the basis of endocrine etiology the condition may be arbitrarily divided into two groups: primary gynecomastia and secondary gynecomastia. In the primary group would fall those types in which a primary hormonal deficiency exists. In the secondary group would fall those types where there is an associated pathology with a primary natural or artificial increase of one or more of the body hormones.

The primary group would include the following: 1) spontaneous gynecomastia of puberty (13); 2) idiopathic gynecomastia without other physical signs of endocrine disturbance (6); 3) cases which develop postoperatively following prostatectomy (13); 4) cases associated with testicular atrophy secondary to disease or trauma (13); 5) a clinical type with endocrine dysfunction characterized by small testes, azoospermia, and an increased excretion of pituitary gonadotrophins.

The phenomenon of breast enlargement is frequently accompanied by defective genitalia, tendency to femininity and lack of sexual desire. Secondary characteristics such as hair growth may be normal. A case of this type was described by Brownstein (3) in 1939. In 1942 Klinefelter, Reifenstein, and Albright (20) described, with complete studies, nine cases of this type characterized by gynecomastia, aspermatogenesis without aleydigism and increased excretion of FSH (follicle-stimulating hormone). These authors felt that in the particular syndrome they described there was normal Leydig cell function and thus normal androgen production. Their studies showed atrophy of germinal epithelium in the testes with aspermatogenesis. The Leydig cells appeared normal or comparatively so in the cases biopsied. They felt the gynecomastia in their series was due to androgen acting on breast tissue in the absence of an X-hormone factor (called "inhibin" by McCullagh and Walsh), which is produced normally by the cells of the seminiferous tubules. They noted that there was a normal or slightly decreased excretion of the 17-ketosteroids. Their conclusion was that a factor of unknown origin caused a selective lesion of the seminiferous tubules resulting not only in aspermatogenesis but also in a lack of X-hormone (inhibin). They postulated that the lack of X-hormone (analo-

gous to estrin in the female), by failing to inhibit the FSH production in the pituitary, leads not only to the increase of FSH but, by failing to stimulate the luteinizing hormone production in the pituitary, to the decrease of LH as well. Consequently, the intact Leydig cells produce somewhat less androgen than normally. In the absence of X-hormone the androgen produced acts on the breast tissue during puberty to cause gynecomastia. As further evidence favoring their theory, they point out that male castrates rarely develop gynecomastia.

These authors did not feel that estrogen substances were in excess in their cases. Estrin levels were determined in two of their patients and found normal.

Heller and Nelson (15), investigating a group of 20 patients with the characteristic triad of aspermatogenesis, increased FSH excretion in the urine and small testes in which biopsy revealed hyalinization of tubules and clumping of the Leydig cells, concluded among other facts that gynecomastia was a variable sign associated with the basic triad. Fourteen of these cases exhibited gynecomastia. In the patients with this breast enlargement the urinary excretion of 17-ketosteroids, representing the androgenic substances of both the adrenals and testicles, was a low normal or subnormal. Four of the five cases with a four plus gynecomastia (using one plus to four plus as an index of involvement) had a normal urinary estrogen excretion. Of the remaining ten cases, the urinary estrogens were low in seven and not determined in three. There was a definite demonstrable rise in the urinary excretion of the pituitary gonadotropins in all cases. Four patients in their series with the most marked gynecomastia were remarkable, because, other than breast enlargement, there was only small evidence of disturbed endocrine dysfunction. These four patients with marked gynecomastia had normal hair distribution, voice pitch, muscular strength, phallus proportions and skeletal development.

These authors in a companion article (16) cited cases of gynecomastia occurring in prepuberal castrates. They felt that some member or precursor of the 17-ketosteroids complex might have mammogenic activity. This substance, the nature of which is admittedly unknown, is produced in the adrenal cortex and appears only, or occurs in increased amounts, under circumstances which have their origin in the failing function of the Leydig cells, with subsequent compensatory activity of the adrenal cortex. They also felt that there was no relation between the levels of excretion of estrogen and the condition of the breasts. However, they disagree with Klinefelter et al. (20), that the testes secrete a non-androgenic hormone, inhibin, which in addition to its action in inhibiting the production of the FSH factor by the hypophysis also inhibits the mammogenic action of testosterone. They felt that there was little evidence for the existence of

inhibin and for a mammogenic activity of testosterone.

The group of secondary gynecomastia, where there is a primary, natural or artificial increase of one of the body hormones, includes the following: (1) After administration of estrogens in carcinoma of the prostate which frequently results in unilateral or bilateral breast hypertrophy (7). (2) Gynecomastia produced, as reported by McCullagh and Rossmiller (21), following testosterone therapy. Pratt (23) reported a case of a typical 32 year old male eunuchoid who was given 150 mg. of methyl testosterone orally. Twenty-four hours later the mammary tree was three centimeters in diameter and the nipples were prominent. (3) Testicular neoplasms, such as teratoma, chorionepithelioma and carcinoma (13, 17). (4) Tumors of the adrenal cortex (10). Several cases have been reported where individuals treated with adrenal cortical extract have developed enlarged breasts. It is believed that excess adrenal cortex secretion suppresses testicular androgen production (3). (5) Part of a syndrome associated with pseudohermaphroditism and a true hermaphroditism (13). (6) Hepatic cirrhosis (9). It is believed the liver normally plays a role in the inactivation or destruction of estrogen. Most writers mention that atrophy of the testes has been present in such cases (9). An explanation of the absence of gynecomastia in acute hepatic necrosis and in the majority of cases of cirrhosis is not yet apparent. (7) Pineal and pituitary tumors (13). (8) Anterior pituitary disturbances (13). (9) Hyperthyroidism (13). (10) Cases of bronchogenic carcinoma in which gynecomastia was present have been reported. In one of the cases studied, normal androgen values were found in the urine with an increase of estrogens (8).

Taylor (27), reviewing the subject of chronic cystic mastitis in the female, which on occasion has a microscopic appearance (26) similar to gynecomastia, failed to reach a definite conclusion regarding the etiology of chronic cystic mastitis. There is some evidence (11) that an endocrine disturbance is the cause of chronic cystic mastitis in the female. It is possible that evidence regarding the cause of the condition in one of the sexes will assist in understanding the similar disturbance in the opposite sex. A theoretical consideration in explanation of the higher incidence of chronic cystic mastitis in the female may be the fact that less disturbance of the hormonal balance is required in the female (as compared to the male) to cause the female's endocrine status to approach the A:E ratio found in gynecomastia. Furthermore, the great monthly cyclic variation of the female's endocrine balance is not present in the male.

When considering the endocrine etiology one must bear in mind the close structural relationship of the estrogens and androgens. The possible conversion of androgen to estrogen or vice versa within the body should not be forgotten (2).

## DIFFERENTIAL DIAGNOSIS

In the differential diagnosis of gynecomastia, three breast conditions one should consider are: 1), infectious processes, 2), increase in the subcutaneous tissue of the breast, and 3), neoplasms (11).

The infectious processes include those of pyogenic, luetic and tuberculous origins.

Geschickter (11) points out that 77 per cent of boys between the ages of 13 and 16 years manifest a button-shaped subareolar node of palpable size. It is the result of hyperplasia of duct epithelium and periductal stroma. The node tends to disappear at about the age of 17 years. Geschickter (11) states it is incorrect to call this node gynecomastia.

The term pseudogynecomastia has been applied to the condition where there is an increase of fat in the mammary region associated with obesity of feminine distribution.

Few of the neoplastic lesions present a clinical picture similar to gynecomastia. These lesions are fibromas, adenomas, lipomas, hemangiomas, cysts, and papillomas. Occasionally sarcomas and carcinomas present a similar picture.

## PATHOLOGY

In Geschickter's (11) series of 108 cases 86 per cent were unilateral. In a review of 71 cases operated on at Brooke General Hospital (31), three cases had bilateral involvement and the remainder were unilateral. The distribution between right and left mammary gland involvement in the latter was equal. It was noted that many of these cases grossly presented a single nodule, but that microscopically they were reported as gynecomastia.

According to Geschickter (11) there are three gross types: 1) the diffuse hypertrophic type seen in early puberty (in such cases the enlargement is usually moderate), 2) the fibro-adenomatous type which is more definitely nodular, and 3), the true feminization of the breast where the size of one or both glands approach that seen in the adult female.

Microscopically, the breast shows "a growth of mammary ducts and periductal stroma. Lobule formation is absent. There is hyperplasia of the duct epithelium with moderate desquamation of cells into the lumen. Small papillary-like projections may form in the lining of the ducts and occasionally the ducts are dilated and contain secretion. A moderate periductal infiltration of wandering cells occurs. There may be an accompanying hypertrophy of the subcutaneous sweat glands. The entire picture may be indistinguishable from the early development of the normal female breast at puberty" (11). In the nodular type almost pure fibrous tissue may be found (11). In some cases (26) the changes are identical with those seen in chronic cystic mastitis in the female.

## THERAPY

The primary etiology should be treated whenever possible. There is also need for treatment of the enlarged gland because of the psychic embarrassment, especially under conditions where large groups of men are living together in total lack of privacy.

"The treatment of gynecomastia resolves itself into two divergent schools of thought: those who believe the great majority of cases will respond to androgens, especially if the condition is bilateral, and those who believe surgery is the method of choice" (26).

Adair (1) feels that unless the condition responds to testosterone it should be considered mastitis rather than gynecomastia, and states further that the best treatment for the condition is hot compresses and scientific neglect. Bronstein and Cassorla (4) believe hormonal therapy with androgenic substances is not indicated where sexual development is adequate. Hoffman, according to Geschickter (11) reported favorable results with 25 milligrams of testosterone intramuscularly two times weekly for ten to twelve weeks. McCullagh and Rossmiller (21), however, have reported the production of breast enlargement following testosterone therapy.

For cosmetic reasons surgery is the treatment of choice, but should be postponed until it is evident that the enlargement will not regress spontaneously (11). Gooel (14) recommends a slightly curved, transverse incision, inferior to the areola, which allows for the excision of the entire breast and offers approach to all positions including the axilla.

## PRESENTATION OF CASES

*Case 1:* A twenty-year-old, unmarried white male had noticed a slight increase in the size of his left breast during February of 1945. There had been a gradual increase in the size of the gland until February 1946 at which time he presented himself at our dispensary. At no time was there any tenderness of the breast, reduction in size or secretion. When he first noticed the enlargement, the soldier thought he had possibly incurred some trauma to the left breast, but was not certain. He had found it unnecessary to shave until eighteen years of age. At present he does not shave more than twice a week. Erection, ejaculation and intercourse had been satisfactory, but a decided lack of interest in the opposite sex was related. Prior to entering the Army he was employed as a farm laborer. After taking 15 weeks of basic infantry training, he was assigned as a vehicle driver which was his assignment when seen by us.

There was no history of jaundice, mumps, orchitis or trauma to the testicles.

After careful studies the involved gland was removed surgically.

*Case 2:* A twenty-year-old, unmarried white male reported on sick call with an enlarged left mammary gland. He had been examined for the above complaint three months earlier, but the enlargement had been questionable at that time. Since then there had been a gradual increase of the breast tissue to the presenting size. At no time was a reduction in size of the involved gland, or a secretion from it noted. Occasionally some

tenderness on pressure had been present. No history of trauma to the breast could be elicited. The history of past illnesses revealed mumps at the age of four with no associated orchitis. No injury to the testicles was remembered and the patient did not believe his testes were ever larger than at the time when first examined by us.

He began to shave at the age of seventeen and at present shaves daily. Erection and ejaculation were reported as having been satisfactory, but intercourse had not been attempted. A greater lack of interest in the opposite sex existed than in Case 1.

For six months after completing high school he was employed as a manual laborer. After taking 21 weeks of basic infantry training, he was assigned as an infantryman for 14 months. During four months prior to the enlargement of his breast he had been assigned as a clerk.

The involved gland was removed surgically after careful studies.

The pertinent physical findings, and laboratory results in the two cases are tabulated in Tables I and II, respectively.

Perirenal air injections (24) for roentgenographic studies were not performed in view of the lack of reason to suspect active adrenal gland pathology and the occasional mortality associated with the procedure.

TABLE I. PHYSICAL EXAMINATION

	Case No. 1	Case No. 2
Age	20	20
Weight	170 pounds	198 pounds
Blood pressure	115/80	140/90
Duration of Gynecomastia	1 year	4 months
Degree of Gynecomastia	Advanced	Moderate
Height	65½ inches	72½ inches
Span	67 inches	72 inches
Body length, pubis to crown	33½ inches	34½ inches
Width of shoulders	17 inches	16½ inches
Width of hip, iliac crest	11½ inches	12½ inches
Width of hip, Fem. trochanter	13 inches	14½ inches
Muscular development	Normal	Normal
Prostate size	½ average	Average
Penis	Normal	Normal
Testes, average size (in cm.)*	5×2.5×3.75	2.5×1.25×1.25
Hair distribution		
Head hair	Heavy	Heavy
Hairline withdrawal	None	None
Facial hair	Sparse	Normal
Axillary hair	Normal	Normal
Body hair	Absent	Normal
Pubic hair	Male	Male
Extremity hair	Absent	Absent

\* Average testicular measurements (4.5 cm. in length, 2.5 cm. in breadth and 3 cm. in the anterior-posterior diameter) (From Gray, Henry, edited by Lewis, Warren H., *Anatomy of the Human Body*, Ed. 24, Philadelphia, Lea and Febiger, 1942, page 1260.)



TABLE II. LABORATORY EXAMINATIONS

	Case No. 1	Case No. 2
Roentgenographic studies		
Skull, abdomen, chest	Normal	Normal
Sella tursica	Normal	Normal
Bone age	Normal	Normal
Excretory urogram	Normal	Normal
Blood studies		
R.B.C., W.B.C., HB., diff.	Normal	Normal
Hematocrit	40% V.P.C.	40% V.P.C.
Sedimentation rate (60 min.)	7 mm.	14 mm.
Blood chemistry		
Urea N. mg. % (whole blood)	4	10
Total blood protein Gms./100 cc.	7.7	7.5
Serum albumin Gms./100 cc.	5.2	5.3
Serum globulin Gms./100 cc.	2.5	2.2
Serum cholesterol	182	174
Cephalin flocculation test	Two plus	Negative
Hippuric acid test (intravenous)	1.0 gm.	1.2 gm.
Icteric index	2.0	2.5
Kahn	Negative	Negative
Glucose tol. (Rose-Exton)	Normal	Normal
Insulin tolerance	Normal	Decreased
Basal metabolic rate	Minus 5%	Minus 7%
Urine examination		
Routine and micro.	Negative	Negative
Bile salts and pigments	Negative	Negative
Urobilinogens mgm. %	1.9	1.7
FSH mouse units/24 hours	Increased	Increased
17-ketosteroids	Normal	Decreased
Estrogens-urinary-mouse units/24 hrs.	Not increased	Not increased
Spermatic fluid examination		
Volume	4.2 cc.	5.0 cc.
Sperm count/cc.—Average	30,000,000	Azospermia
Spermatozoa		
Motility	50% amotile	—
Morphology	Normal	—
Microscopic diagnosis—breast	Gynecomastia	Gynecomastia

## DISCUSSION OF CASES

Both cases had diffuse enlargement of the mammary gland resembling the female breast at puberty, which is considered to be representative of true gynecomastia. The microscopic picture of the breast was typical in each case.

In Case 1 the only significant finding beside the breast enlargement was

the persistent oligospermia averaging 30,000,000 spermatozoa per cubic centimeter. The two plus cholesterol-cephalin flocculation test could not be explained. In the absence of any other marked deviation from normal in either physical or laboratory examinations, it was difficult to make a specific conclusion as to the etiology of the gynecomastia in this case. The soldier's sparse beard, admitted absence of libido and deficient spermatogenesis indicated a possible endocrine factor; although the 17-ketosteroids,



FIG. 1. *Case 1* before surgery.  
(Photo by U. S. Army Signal Corps.)

and estrogen urinary excretion were within normal limits, the FSH excretion was considerably elevated.

We felt this case might be in an early stage of the syndrome first described by Klinefelter's (20) group and later by Heller and Nelson (15). Another consideration was a delayed maturation of the patient from a prolonged puberal stage, in which case the breast enlargement would fall into the group of spontaneous gynecomastia of puberty, which had failed to recede. A less satisfactory third classification would be to label the case as an idiopathic gynecomastia.

Case 2 was a cunuchoid type individual evidenced by the disproportion of body length (29),  $34\frac{1}{4}$  inches (crown to pubis), to the lower extremity length,  $38\frac{1}{4}$  inches (pubis to sole), the subcutaneous fat distribution and atrophic testes. He was classed as a functional prepubertal castrate by us. The history of mumps may have significance as regards the atrophic testes.

However, as mentioned in the case presentation he did not remember any involvement of his testes, had no history of trauma to his testes and no memory of his testes being larger than when examined by us. His markedly atrophic testicles along with the azoospermia, increased FSH excretion,



FIG. 2. *Case 2* before surgery.  
(Photo by U. S. Army Signal Corps.)

decreased 17-ketosteroids excretion and normal estrogen excretion caused us to consider this case as belonging to the group of cases described by both Klinefelter et al. (20) and Heller and Nelson (15).

#### SPECIAL LABORATORY PROCEDURE

The urinary gonadotropins were determined in the manner described by Klinefelter, Albright and Griswold (19). This procedure involves the use of 21-day-old virgin female white mice weighing not more than 6-10 grams when sacrificed—preferably less than 9 grams. The gonadotropins in the urine were extracted and concentrated by the non-dialysis method described by the above mentioned authors. A total quantity of 2.5 cubic centimeters was injected into each mouse in appropriate dilutions. A uterine weight of 7 milligrams or above after the uterus was blotted dry was the criterion for establishing a positive mouse test. However, primary changes in the ovaries and secondary changes of the uterine tubes were also examined for evidence of gonadotropic action. To avoid possible error

in calculating the number of gonadotropic mouse units excreted in 24 hours, the entire 24-hour urine excretion was collected, mixed thoroughly and a 90-minute aliquot used for the tests. An attempt was made to standardize the "blotting dry" of the uterus by placing the uterus between two pieces of filter paper and allowing a 100 gram weight to rest on the uterus for 60 seconds.

The test apparently is chiefly a test of the FSH (follicle stimulating hormone) in the urine. The daily male excretion of FSH is not less than 6.6 mouse units and not more than 96 mouse units per 24 hours (19). The results of tests in the cases presented in this paper and the controls are expressed in Table III.

TABLE III. GONADOTROPINS

Case No.	Av. Wt. of Mice at Sacrifice (Gm.)	Average Uterine Weight of mice (mg.)				Uterine Tubes (engorgement and edema)				Ovaries of Mice (Evidence of follicle maturation)			
		No urine inject	For 96 u. per 24 hr.	For 144 u. per 24 hr.	For 192 u. per 24 hr.	No urine inject	For 96 u. per 24 hr.	For 144 u. per 24 hr.	For 192 u. per 24 hr.	No urine inject	For 96 u. per 24 hr.	For 144 u. per 24 hr.	For 192 u. per 24 hr.
Control	7.8	8.84	5.76	—	—	Neg.	Neg.	—	—	Neg.	Neg.	—	—
1	7.1	—	7.2	8.8	7.9	—	Neg.	Neg.	Neg.	—	Neg.	Neg.	Neg.
2	7.4	—	7.6	6.0	6.3	—	Pos.	Pos.	Neg.	—	Neg.	Neg.	Neg.

Note: According to Klinefelter, Albright and Griswold (19), the upper limit of normal male FSH (Follicle Stimulating Hormone) excretion is 96 units per 24 hours.

It was noted that mice receiving no urine injections had uterine weights consistently above the uterine weights of those mice which received injections of urine from the individuals who were apparently normal endocrinologically. These concentrates were prepared by the nondialysis method (19), used for all our FSH tests. No mice used in the tests died from the urine injections. As in all biological tests, one or two mice are not a reliable index for any given reading.

The urinary estrogens were determined on 21-day-old female white mice using aqueous solutions made from the ether precipitates of aliquots of total 24-hour urine specimen. The uterine weight increase in the mice, as used by Scott and Vermeulen (25), was the criterion for evidence of estrogenic action. Uterine weights above 7 milligrams were considered to be a positive mouse test. The uterine tubes were also examined for evidence of estrogenic action. These findings were compared to similar determinations made from aliquots of normal male 24-hour urine specimens. The results of these tests are expressed in Table IV.

The 17-ketosteroids were determined on a portion of a 24-hour urine

TABLE IV. ESTROGENS

Case No.	Av. Wt. of Mice at Sacrifice (Gm.)	Average Uterine Weight of mice (mg.)				Ovaries of Mice (Evidence of follicle maturation)			
		For 8 u. per 24 hr.	For 16 u. per 24 hr.	For 64 u. per 24 hr.	For 8 u. per 24 hr.	For 16 u. per 24 hr.	For 16 u. per 24 hr.	For 32 u. per 24 hr.	For 64 u. per 24 hr.
Control	6.6	6.6	5.1	5.7	5.9	6.9	Neg.	Neg.	Neg.
1	7.0	11.6	7.8	5.4	5.3	Neg.	Pos.	Neg.	Neg.
2	6.5	—	12.9	6.6	—	—	Neg.	Neg.	—

specimen by the Pincus colorimetric assay\* as described by Cahen and Salter (5). The normal values for the 17-ketosteroids given for the method are 8 to 23 milligram-equivalents of androsterone per 24 hours. The results are shown in Table V.

TABLE V. 17-KETOSTEROIDS

	Normal Values	Case No. 1	Case No. 2
17-Ketosteroids excretion (mg. per 24 hr.)	8-23	12.6	5.9

The 17-Ketosteroids are expressed in terms of milligram-equivalents of androsterone.

### COMMENT

The numerous theories put forth concerning the etiology of male breast enlargement are difficult to evaluate in view of the present comparatively limited knowledge of the endocrine functions occurring in the body. That trauma can be a factor in some cases must be admitted. The "lack of inhibin" theory of Klinefelter et al. (20) is worth considering and not without basis. Heller and Nelson's (15) proposal of an abnormal mammogenic 17-ketosteroid being the cause of gynecomastia requires further experimental evidence. In our opinion the altered A:E ratio is a constant factor in the cases reviewed. The fact that the 17-ketosteroids urinary excretion was consistently low or sub-normal in most of the cases of Klinefelter et al. (20) and also those of Heller and Nelson (15) lends further credence to this conception. It is also to be noted that the spontaneous gynec-

\* The androsterone for the 17-ketosteroid colorimetric assay was supplied through the courtesy of the Ciba Company, Summit, New Jersey.

mastia of puberty is associated with a period of radical alteration of the production of androgens and estrogens in the body.

One of the most constant factors encountered by Klinefelter et al. and Heller and Nelson was the persistent elevation of gonadotropins. This increase is believed to be the result of the removal of the normal inhibiting influence of the testicular hormone, testosterone. The gonadotropins are also believed to increase at the time when the spontaneous gynecomastia of puberty occurs. A possible mammary gland stimulating action of the gonadotropins in the presence of androgen may be considered.

A final observation on the phenomenon is that while functional pre-pubertal castrates develop gynecomastia occasionally, complete surgical castrates apparently do so rarely. The majority of patients with gynecomastia evidently show varying degrees of reduction of testicular function. Clinical observations (21, 23) tend to substantiate the idea that a certain level of androgenic substance must be present in the body before gynecomastia can develop.

#### SUMMARY

Two cases of gynecomastia were presented with laboratory data, and the following points were discussed: (A) Incidence; (B) Etiological factors; (C) The endocrine disturbances possibly responsible for the condition; (D) The pathology and occasional similarity of the microscopic picture of gynecomastia to that of chronic cystic mastitis in the female; (E) The necessity for treatment of gynecomastia because of the mechanical irritation and psychic embarrassment; (F) The hormonal therapy and surgical removal of the involved breast in gynecomastia.

#### CONCLUSION

Gynecomastia is the result of a disturbed endocrine status usually related to an alteration of the normal androgen:estrogen ratio within the individual. In those patients where no cause for the phenomenon can be ascertained, our failure is probably due to the inadequacy or incompleteness of present methods of examination. If possible the primary cause should be treated; when impossible, the present therapy of choice is surgical removal of the enlarged gland.

#### ACKNOWLEDGEMENT

We gratefully acknowledge the cheerful assistance of Miss Addelia Peterson and Major William F. Hettler of the United States Army Sanitary Corps in performing the special laboratory tests required for our studies. We also wish to express our appreciation for the use of the facilities of the United States Army Eighth Service Command laboratory, Fort Sam Houston, San Antonio, Texas.

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31. From pathology reports, Brooke General Hospital Laboratory, Fort Sam Houston, Texas.
32. From Surgical Service, Brooke General Hospital, Fort Sam Houston, Texas.





# ANNOUNCEMENT OF THE 1948 MEETING OF THE ASSOCIA- TION FOR THE STUDY OF INTERNAL SECRECTIONS

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The Thirtieth Annual Meeting of the Association for the Study of Internal Secretions will be held in the Palmer House, Chicago, Illinois, June 18 and 19, 1948.

The scientific sessions will be held in the Red Lacquer Room and registration will be on the fourth floor just outside the Red Lacquer Room. The Annual Dinner will be held in the same room on Friday, June 18th at 7 p. m. and will be preceded by a cocktail party, the location of which will be announced later. The Council will meet at 2 p. m. Thursday, June 17th.

All members of the Association who plan to attend the Thirtieth Meeting are urged to make their reservations at once with the Palmer House, stating the time of arrival and how long they plan to remain in Chicago.



# Announcement of Award

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## AMERICAN ASSOCIATION FOR THE STUDY OF GOITER VAN METER PRIZE AWARD

The American Association for the Study of Goiter again offers the Van Meter Prize Award of Three Hundred Dollars and two honorable mentions for the best essays submitted concerning original work on problems related to the thyroid gland. The Award will be made at the annual meeting of the Association which will be held in Toronto, Canada, May 6th, 7th, 8th, 1948 providing essays of sufficient merit are presented in competition.

The competing essays may cover either clinical or research investigations; should not exceed three thousand words in length; must be presented in English; and a typewritten double spaced copy sent to the corresponding secretary, Dr. T. C. Davison; 207 Doctors Building; Atlanta 3, Georgia not later than February 1st, 1948. The committee, who will review the manuscripts, is composed of men well qualified to judge the merits of the competing essays.

A place will be reserved on the program of the annual meeting for presentation of the Prize Award Essay by the author if it is possible for him to attend. The essay will be published in the annual Proceedings of the Association. This will not prevent its further publication, however, in any Journal selected by the author.

T. C. DAVISON,  
*Corresponding Secretary*

### OBITUARY

Ernst Laqueur, 68, Professor of Pharmacology at the University of Amsterdam, Holland, died on August 19, 1947, while vacationing in Switzerland.

He was the discoverer of testosterone which name he coined. He also did fundamental work on the standardization of insulin and estrogens and belonged to the Committee of the League of Nations which established the International Standards of estrone and estradiol benzoate. He inaugurated the clinical use of androgens in prostatic hypertrophy and treated Cushing's syndrome successfully with massive doses of estradiol benzoate.

In 1946 he was awarded the Berzelius Medal by the Swedish Medical Society.

Doctor Laqueur founded the *Acta Brevia Neerlandica* and was one of the originators of the *Excerpta Medica*, the publication of which is just starting.

# Abstracts of

## CURRENT ENDOCRINE LITERATURE

*Editor*; D. A. McGINTY. *Collaborators*: A. R. ABARBANEL, F. N. ANDREWS, B. L. BAKER, F. A. DE LA BALZE, ISRAEL BRAM, R. A. CLEGHORN, RUCKER CLEVELAND, C. D. DAVIS, ANNA FORBES, M. B. GORDON, H. S. GUTERMAN, M. M. HOFFMAN, R. G. HOSKINS, C. D. KOCHAKIAN, H. S. KUPPERMAN, H. L. MASON, JANET W. MCARTHUR, THOMAS H. MCGAVACK, A. E. MEYER, K. E. PASCHKIS, A. B. PINTO, J. R. REFORZOMEMBRIVES, E. C. REIFENSTEIN, JR., G. G. RUDOLPH, L. T. SAMUELS.

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### ADRENALS

THELANDER, H. E. Congenital adrenal-cortical insufficiency associated with macrogenitosomia. *J. Pediat.* 29: 213 (1946).

The author reports the interim story and final study of a case of congenital adrenal-cortical insufficiency associated with macrogenitosomia which he had reported previously (*J. Pediat.* 18: 779 (1941)). This boy was studied for six years to the time of his death with measles. The complete case history and findings at post-mortem examination are given. A table is provided of weights and measurements taken at about yearly intervals. It was estimated that, if the boy had lived, his ultimate height would have been about 5 feet, in other words below average. His teething corresponded with his chronologic but not with his physiologic age. The facial contour, voice, appearance, and distribution of body hair were that of an adolescent; the testicles were small and undeveloped. Mentally and emotionally the child did very well. At various times the patient received whole adreno-cortical extract, adrenocorticotrophic hormone, and particularly desoxycorticosterone acetate. This latter medication was administered sub-lingually in a dosage of  $\frac{1}{2}$  mg. (2 drops) four times a day. This dose was subsequently increased to five times daily, and finally to 4 drops five or six times a day. When the dosage was reduced, he was restless and irritable, had a poor appetite, and drank excessive amounts of water; however, he had no craving for salt. The presence of androgenic hormones was best illustrated in the growth of the child who was husky and energetic. At post-mortem he was found to have bilateral adreno-cortical hyperplasia which was extreme, and he had aberrant adreno-cortical rests in both testes. He also had a persistent thymus, but aside from this the organs were normal. In spite of the extreme rapidity of his growth, the striated muscle of the body and the muscles of the heart were normal, and the bony structure, teeth and organs were well formed. There is no evidence of lack of mineralization or avitaminosis. There was a family history of disease involving the adrenal cortex, since a sibling who died with somewhat similar symptoms, probably had hypospadias and was presumably a female pseudohermaphrodite.—*E.C.R., Jr.*

### TESTES

DELORY, G. E. Seminal fluid acid phosphatase in sterility. *Brit. M. J.* 1: 566 (1947).

Samples of seminal fluid from 36 men being investigated for sterility were examined

for acid phosphatase, pH, morphology, motility, and abundance of spermatozoa. No correlation was found between the enzyme concentration and either the number of spermatozoa or their motility. There was no evidence that the acid phosphatase is consistently low in male sterility.—*L.T.S.*

MACLEOD, J. AND R. S. HOTCHKISS. Semen analysis in 1,500 cases of sterile marriages. *Am. J. Obst. and Gynec.* 52 (1): 34-41 (1946).

Deficiencies either in spermatozoa count, motility or morphology, were found in about 50 per cent of 1,500 patients who had semen analysis because of presumed sterility. The authors found that as the spermatozoa count fell below 60 million per cubic centimeter other defects, such as motility and abnormal morphology, became more apparent, until in the very low count range all three deficiencies together are likely to be found. It is suggested that the 60 million per cubic centimeter spermatozoa count level is a reasonably dividing line between good and impaired fertility.—*C.D.D.*

## OVARIES

AYRE, J. E. AND W. R. FOOTE Granulosa-cell tumor with pregnancy following removal. *Am. J. Obst. and Gynec.* 51 (2): 260-264 (1946).

The authors report a granulosa-cell tumor of the ovary in a 35-year old woman who had had amenorrhea for four years prior to operation. Menstruation returned promptly following operation. Fertilization occurred, and the resulting pregnancy carried on normally to maturity.—*C.D.D.*

BIANCO, J. J. AND G. O. FAVORITE. Granulosa-cell tumor of the ovary. *Am. J. Obst. and Gynec.* 52 (4): 677-680 (1946).

A case report is presented of a granulosa cell tumor occurring in a 55-year old woman.—*C.D.D.*

CHESLEY, J. C., S. A. COSGROVE AND J. PREECE. Hydatidiform mole, with special reference to recurrence and associated eclampsia. *Am. J. Obst. and Gynec.* 52 (3): 311-320 (1946).

The authors found 40 instances of recurrent hydatidiform moles and only 5 of these women had normal pregnancies occurring between moles. Thirty-five instances of probable or alleged eclampsia in conjunction with hydatidiform mole were encountered. In over 75,000 deliveries, 57 hydatidiform moles were found, an incidence of one in 1,321 deliveries. Three of these moles were followed by chorionepithelioma.—*C.D.D.*

CURTIS, A. H. The origin of adrenal-like tumor of the ovary. *Am. J. Obst. and Gynec.* 52 (1): 115-122 (1946).

A case report is presented of an adrenal-like tumor of the ovary in a 23 year old girl. This is the sixteenth such case to be reported in the literature. The author demonstrates in photomicrographs a gradual transition from a polygonal adrenal type of cell to a stroma composed of spindle-shaped cells. The author believes that study of this tumor makes it apparent that adrenal-like tumors of the ovary arise by metaplasia of stroma cells or other indifferent cells, rather than from embryologically misplaced tissue in the ovary.—*C.D.D.*

CURTIS, A. H. Another case of arrhenoblastoma. *Am. J. Obst. and Gynec.* 52 (1): 128-131 (1946).

The patient was a 52-year old woman who had had signs and symptoms of masculinization for six months prior to surgery. No hormonal assays are reported. Postoperatively the voice modulated, the hypertrichosis largely disappeared and the clitoris returned to normal size. The length of the followup period is not stated.—C.D.D.

FARBER, E. P. The induction of labor with methergine: preliminary report. *Am. J. Obst. and Gynec.* 51 (6): 859-865 (1946).

A new synthetic ergot preparation was employed in the medical induction of 43 consecutive patients, and satisfactory results were obtained in 39 instances. The reported dosage was 0.5 cubic centimeter given orally in about an ounce of water and then 1 cubic centimeter every 30 minutes for 4 doses. There were no untoward effects noted on mother or fetus.—C.D.D.

FARRIS, E. J. A test for determining the time of ovulation and conception in women. *Am. J. Obst. and Gynec.* 52 (1): 14-27 (1946).

Observations were made on 100 women, 12 unmarried and 88 married. Two cubic centimeters of a voided morning specimen of urine was injected subcutaneously into 2 immature Wistar rats ranging between 22 and 25 days of age, and weighing between 30 and 50 grams. The animals were sacrificed in 2 hours and the degree of redness of the ovaries was compared with the colors of the Munsell color chart. The normal positive reaction consists of a definite deepening of the ovary of the rat for 3 or 4 consecutive days near the middle of the menstrual cycle. The abnormal reactions include no color, one or two days only of color, or a split reaction—one or two days of color separated by no color for one, two or more days. Urine assays showed gonadotropin present when the hyperemic reaction was positive. The author believes that, on the basis of artificial insemination studies, conception is most apt to occur on the third or fourth day of a normal reaction.—C.D.D.

FOOTE, E. C. AND C. E. SEEGAR JONES. An evaluation of the Hogben pregnancy test. *Am. J. Obst. and Gynec.* 51 (5): 672-677 (1946).

An analysis of 157 Hogben pregnancy tests is reported in the following conditions: 60 intrauterine pregnancies, 11 abortions, 2 ectopic pregnancies, 1 abdominal pregnancy and 68 nonpregnant patients with miscellaneous diagnoses. No false positive tests were obtained. The test is apparently not accurate before the fortieth day of gestation. The authors have a 95 per cent accuracy with this test and believe it compares favorably with the Friedman test in accuracy.—C.D.D.

GOLDSTINE, M. T. Arrhenoblastoma of the ovary: report of two cases. *Am. J. Obst. and Gynec.* 52 (1): 123-127 (1946).

The author presents brief case reports of two young patients, 25 and 20 years of age, in whom signs and symptoms of masculinization led to the surgical removal of arrhenoblastomas. Both had uneventful recoveries. Both had return of normal ovarian function. One delivered a full term normal infant 19 months after surgery and the other was at the time of this report in her thirty-second week of a normal pregnancy 14 months following surgery.—C.D.D.

HAMBLEEN, E. C. Some contributions of endocrinology to obstetrics and gynecology. *Am. J. Obst. and Gynec.* 51 (6): 796-803 (1946).

A few of the significant endocrine contributions to obstetric and gynecologic thought and practice have been surveyed. It is not too much to hope that the next fifty years may bring many further endocrine solutions to physiologic, diagnostic and therapeutic problems of our specialty. It may well be that the ultimate solution of eclamptogenic toxemia and genital carcinoma may be effected through the medium of an expanding knowledge of the endocrine system (Author's Summary).—C.D.D.

HARDING, F. E. The oral use of hexestrol for estrogen deficiency. *Am. J. Obst. and Gynec.* 51 (5): 660-665 (1946).

Ninety patients were treated with oral hexestrol in average daily doses of 1 to 2 milligrams. Thirty-two of these were in the climacteric group, but 16 were still menstruating, 3 regularly. Twenty-five had artificial menopause, but 6 of these had only subtotal hysterectomies. The remaining 33 patients had other conditions with a suspected ovarian deficiency. The author reports that all 90 patients were treated with hexestrol with satisfactory results. A low incidence of toxic reactions (3 to 6 per cent) is reported. It is reported that there were less undesirable effects upon uterine bleeding with hexestrol than with conjugated estrogens—equine, diethylstilbestrol propionate and ethinyl estradiol.—C.D.D.

HAUCK, H. M. Plasma levels and urinary excretion of ascorbic acid in women during the menstrual cycle. *J. Nutrition*, 33 (5): 511-515 (1947).

The author studied the plasma levels and urinary excretion of ascorbic acid relative to the menstrual cycle. The subjects were 10 women who were maintained on constant amounts of ascorbic acid for periods of 4-6 weeks. No unusual variations in the plasma levels or excretion of the vitamin were found when mid-cycle values were compared with those obtained on the first day of menses.—H.S.G.

JONES, G. S. AND H. S. EVERETT. Arrhenoblastoma of the ovary, with a report of two cases. *Am. J. Obst. & Gynec.* 52 (4): 614-622 (1946).

The authors report 2 patients with a pathological diagnosis of arrhenoblastoma. One had no bleeding for 8 months, but little else except considerable hirsutism to suggest masculinization. The other had definite masculinization including hoarseness and enlargement of the clitoris. In the latter patient, preoperatively the urinary 17-ketosteroids were 56 and 36 milligrams per 24 hours on two separate occasions, and 12 days postoperatively the level had fallen to 11 milligrams per 24 hours. No pregnanediol was present in the urine. This tumor was composed almost entirely of interstitial cell elements with only occasional tubular formation.—C.D.D.

LUBIN, S. The routine use of stilbesterol for engorgement and lactation in nonnursing mothers. *Am. J. Obst. and Gynec.* 51 (2): 225-229 (1946).

One hundred postpartum patients who did not nurse their babies were studied as follows: One-half the group served as controls. The other half were given oral diethylstilbesterol, 10 milligrams, three times daily for two days, followed by 5 milligrams three times daily until the patient was discharged from the hospital. This regime was started

within the first 24 hours after delivery, and patients were observed for pain, engorgement, milk, fever and erythema of the breast. Pain was absent in 58 per cent of the control group and 88 per cent of the treated group. Milk secretion was affected as follows: control group—leakage, 76 per cent and expressed milk, 24 per cent; diethylstilbesterol group—leakage, 34 per cent and expressed milk, 56 per cent. The author points out that 50 per cent of the treated group developed full, painful breasts at some time within two weeks after leaving the hospital. He concludes that while the oral administration of diethylstilbesterol will prevent or postpone engorgement of the breast and its attendant pain, the advisability of its routine use in nonnursing mothers is questionable.—C.D.D.

LYON, R. Pregnanediol excretion at the onset of labor *Am. J. Obst. and Gynec.* 51 (3): 403-410, (1946).

The author studied sodium pregnanediol glycuronide levels in the late antepartum and postpartum periods of premature, normal and postmature deliveries. The titrimetric method of Allen and Viergiver, with a modification of extraction technique suggested by Woolf, Viergiver and Allen, was employed. The composite of 68 assays, in patients who delivered spontaneously, showed the following levels: 5 days antepartum, 30 mg; 2 days, 19.5; 1 day, 14.8; the day of delivery 12.5; first day postpartum, 8.8 and second day 4.6 mg. of sodium pregnanediol glycuronide per 24 hours. There were no marked differences in values whether the delivery occurred prematurely, normally, postmaturely or by cesarean section. The author believes his data implies that the concentration of progesterone available at the onset of labor is insufficient to maintain and continue pregnancy.—C.D.D.

MCCORMACK, G. A comparison of the color chemical test with the Friedman modification of the Aschheim-Zondek test. *Am. J. Obst. and Gynec.* 51 (5): 722-725 (1946).

The chemical color reaction test for pregnanediol was done simultaneously with the Friedman test on 304 patients. Of this number, 262 were in agreement. Negative chemical tests and positive Friedman tests were obtained on 38 patients diagnosed as threatened abortion. Two instances of hydatid mole were tested. In both instances, the chemical tests were negative and the Friedman positive. Corpus luteum cysts were apparently encountered twice with the anticipated results in assay being obtained. The author believes the two tests have an equivalent degree of accuracy and points out that their combined use can be helpful in establishing certain diagnoses.—C.D.D.

MENDEL E. B. Chiari-Frommel Syndrome: a historical review with case report. *Am. J. Obst. and Gynec.* 51 (6): 889-892 (1946).

A case report is presented. One and one-half years after the birth of the baby, uterine bleeding returned more or less regularly, but intermittent secretion of watery fluid from breasts continued for 2 years. No further pregnancies occurred but a secretory endometrium was found on one occasion.—C.D.D.

MORROW, A. G. AND R. S. BENUA. An evaluation of the Guterman pregnancy test. *Am. J. Obst. & Gynec.* 51 (5): 685-691 (1946).

The authors modified Guterman's original technique by substituting 1 cubic centimeter of 20 per cent solution of sodium in 95 per cent ethanol for 10 cubic centimeters of 2 per cent sodium hydroxide in absolute methanol, and by substituting Number 42

Whatman filter paper instead of a fritted glass filter. With this altered technique, several falsely positive results were obtained during the luteal phase of the menstrual cycle.

The authors point out that the test is not reliable in the presence of functioning corpus luteum tumors and probably also in association with an arrhenoblastoma.—*C.D.D.*

RAKOFF, A. E. Studies on high dosage progesterone therapy of amenorrhea. *Am. J. Obst. and Gynec.* 51 (4): 480-490 (1946).

The author studied 51 patients, 7 of whom had primary amenorrhea and 44 had secondary amenorrhea. The duration of the latter varied from 2 to 13 years in 19, 1 to 2 years in 5, 6 months to one year in 14 and less than 6 months in 6 patients. Initial therapy generally consisted of intramuscular injection of 20 milligrams of progesterone on each of three consecutive days. If no bleeding occurred, priming with small amounts of oral estrogens was employed and the series of progesterone injections repeated. None of those with primary amenorrhea had bleeding after progesterone alone. Induced bleeding occurred in 5 who were primed with estrogens and then were given the progesterone. Sixty-six per cent of those with secondary amenorrhea responded to progesterone alone. The remainder had bleeding following the dual therapy. The most interesting part of the report concerns 2 of the patients with primary amenorrhea. These were of short stature, had sexual infantilism and high gonadotropins. More and more instances of this syndrome are being recognized.—*C.D.D.*

ROSSMAN, I., AND G. W. BARTELMEZ. Delayed ovulation, a significant factor in the variability of the menstrual cycle. *Am. J. Obst. and Gynec.* 52 (1): 28-33 (1946).

A review is presented of the variability in the length of the pre- and postovulatory phases of the menstrual cycle in the macaque. The author reports ovulation on the 21st to the 23d, 29th, 29th to the 30th, 30th and 36th days after the onset of normal menstrual periods in a colony of healthy mature macaques. Delayed ovulation in these instances was due to a postmenstrual period of ovarian inactivity. The author believes such delayed ovulation may be relatively frequent in occurrence.—*C.D.D.*

RUTHERFORD, R. N. Preconceptional progestin therapy in habitual abortion. *Am. J. Obst. and Gynec.* 51 (5): 652-659 (1946).

A series of 63 patients, each of whom had had 3 or more successive abortions, were treated with 5 milligrams of progesterone intramuscularly every other day from the eighteenth day until the menstrual flow supervened. If the period was missed, the injections were continued as before until the beginning of the fourth month and then every third day until fetal motion was felt.

Of the 54 patients who had had 3 successive abortions, 31 delivered viable infants and 28 survived, a corrected percentage of 51.8. Of those who aborted, there were proved pathologic ova in 69.1 per cent. Of the 9 patients who had had 4 successive abortions, 4 carried pregnancies to viability and successful termination. Three of the 5 abortions were associated with pathologic ova.—*C.D.D.*

SMITH, O. W., G. V. S. SMITH AND D. HURWITZ. Increased excretion of pregnanediol in pregnancy from diethylstilbesterol with special reference to the prevention of late pregnancy accidents. *Am. J. Obst. and Gynec.* 51 (3): 411-415 (1946).



A brief resume is presented of the hypothesis that the premature deficiency in the production of estrogen and progesterone characteristic of accidents of late pregnancy might be prevented by the oral administration of diethylstilbesterol. A diabetic woman with a poor obstetrical history was treated with diethylstilbesterol continuously, except for two short intervals, from the sixteenth through the thirty-fifth week, and weekly urinary pregnanediol levels were studied. The levels of pregnanediol fell precipitously whenever the treatment was interrupted and the pregnancy went to term uneventfully.

The authors propose a trial of oral diethylstilbesterol as a preventive measure in patients with a history of repeated accidents of pregnancy which may be referable to progesterone deficiency, namely, abortion, premature delivery, pre-eclampsia, eclampsia, or intrauterine death.—C.D.D.

VIERGIVER, E. AND W. T. POMMERENKE. Cyclic variations in the viscosity of cervical mucus and its correlation with amount of secretion and basal temperatures. *Am. J. Obst. and Gynec.* 51 (2): 192-200 (1946).

Daily observations of basal body temperature and the viscosity of the cervical mucus were made on four normal young women. One was followed through two cycles and the others through four. The term viscosity is defined as the time in seconds required to draw a column of mucus through the capillary tube a given distance at a given pressure, as measured by a mercury manometer. In two of these cycles, the correlation between viscosity and penetrability was also studied. The method of Lamar, Shettles and Delfs was used in determining the penetrability. The rate of migration of the spermatozoa through a capillary tube filled with cervical mucus was estimated by means of a microscope, stop watch and calibrated mechanical stage. These studies appear to indicate that in normal subjects the ovulatory process is characterized by a period of increase in secretion and a decrease in the viscosity of the cervical mucus, along with a shift in the basal temperature.—C.D.D.

WINSON, S. G. An analysis of 257 cases of sterility. *Am. J. Obst. & Gynec.* 52 (4): 631-634 (1946).

Of 257 patients studied, 53 had secondary sterility, and 204 primary sterility. A cervical factor was found in 38 per cent and following local treatment 75 per cent of these conceived. Ninety patients had partial and 50 total tubal occlusion. This was relieved and conception followed in 48 and 44 per cent of the patients respectively. Endocrine menstrual disturbances were found in 134 patients. A surprisingly large number of these, 82 or 31.9 per cent of the entire group, had anovulatory menstruation and 50 percent of these patients conceived. After proper treatment conception took place in 50 per cent of the 257 cases studied. There were 7 abortions and 1 ectopic pregnancy after treatment with equine gonadotropin or chorionic gonadotropin plus "pituitary synergist." The male factor was considered responsible in 40 per cent.—C.D.D.

WINTHER, N. Menorrhoeal problems in college women. *Am. J. Obst. & Gynec.* 52 (5): 803-809 (1946).

In a 10 year study, 5,210 women were seen in the gynecological department of the University of Minnesota Student Health Service because of pain, vaginal discharge and irregular uterine bleeding. Thirty-two per cent of these presented themselves because of irregular bleeding. A brief resume of various types of treatment for menstrual irregularities is given.—C.D.D.

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## THE FACTOR OF PREVIOUS TREATMENT IN EXPERIMENTAL MENSTRUATION<sup>1</sup>

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**A**N EXPERIMENTAL study of the disorders of menstruation has been in progress in our laboratory since 1929. The approach has been along two lines: 1) study of the changes in endometrial histology associated with abnormal uterine bleeding and 2) experimental production of abnormal menstruation in monkeys and direct observation of the associated vascular phenomena. Among other things, the study has demonstrated that menstrual disorders result from lesions in any of the endocrine glands or from constitutional disease secondarily affecting the endocrine system. The primary lesion operates through the ovaries to produce the menstrual symptom. The degree of ovarian involvement is indicated by the endometrial histology. There is no constant correlation between the type of menstrual abnormality and the severity of the ovarian dysfunction as indicated by the endometrium (Burch and collaborators, 1937; 1942 (1, 2)).

The study of endometrial vascular phenomena was undertaken in the hope of clarifying the question of the relationship of the bleeding abnormalities to ovarian failure. This report is one of a series dealing with various aspects of the study.

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## TYPES OF EXPERIMENTS

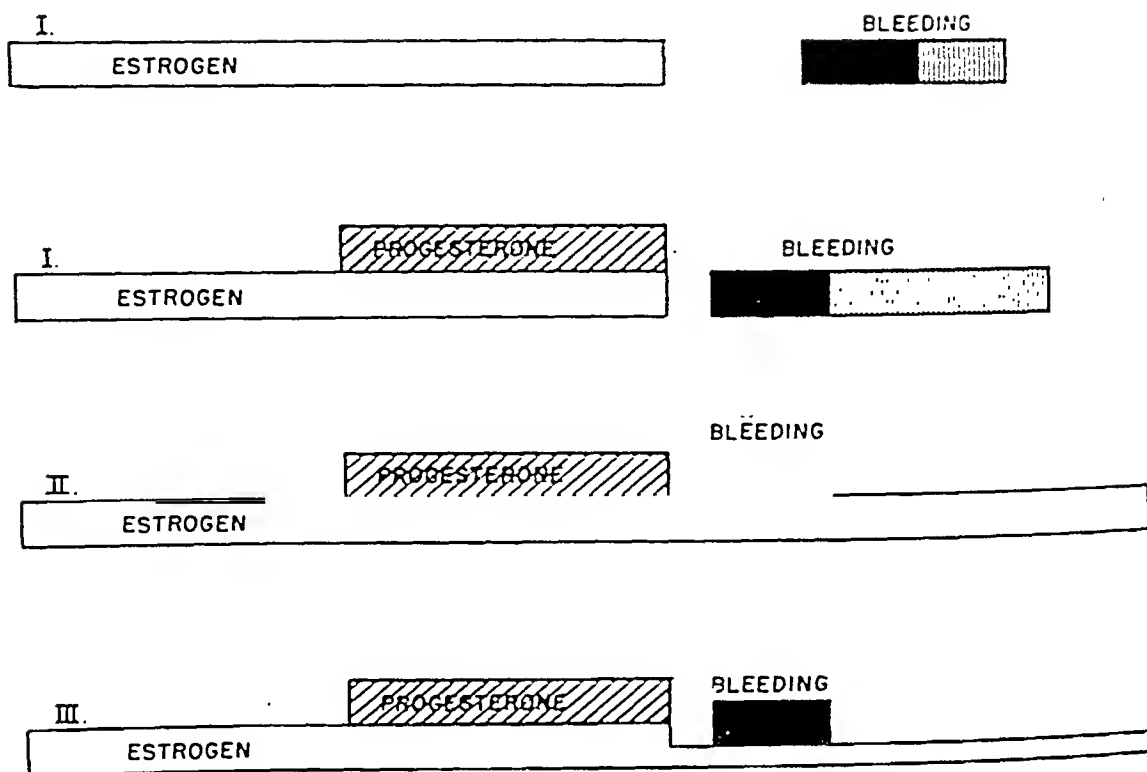


FIG. 1. Types of experimental treatment employed in the present study. Each bar represents one type of treatment. The width of the bar indicates the relative magnitude of the daily dose. The narrow bars indicate dosages within the 'threshold' range (Zuckerman, 1937), the wide bars, dosages above this range. Relative duration of treatment, latent period and uterine bleeding is indicated horizontally. The bars have no specific quantitative significance. The division of bars representing bleeding into black and lined portions is intended to indicate that bleeding produced by the respective types of treatment was sometimes of normal duration, sometimes prolonged.

In a previous report (Phelps, 1946a (5)) evidence was presented which indicates that uterine bleeding is not controlled by any one hormonal influence but by a combination of influences. The factors which may affect any given episode of bleeding can be classified in three categories: 1) the current hormonal stimulus, i.e., the course of stimulation that produces the bleeding, 2) conditions existing prior to application of the current stimulus and 3) the stimulus acting subsequent to the onset of flow.

The factors comprising the first category, the current hormonal stimulus, were discussed in the previous report (Phelps, 1946a (5)). The present report deals with conditions existing prior to application of the current stimulus, the purpose being to present evidence concerning the nature of endocrine factors comprising this category and the mechanism through which their influence upon uterine bleeding is exerted.

The method employed in the study has been described elsewhere (Phelps, 1946 b (6)). Briefly, it consisted of inducing menstruation in ovariectomized rhesus monkeys by injecting ovarian hormones, recording the amount and duration of the uterine bleeding produced and observing the associated vascular phenomena by means of intraocular endometrial transplants (Markee, 1940 (4)). One hundred seven episodes of uterine bleeding were produced in nine monkeys. Hormone dosages employed and duration of treatment varied (cf. table 5). Duration of the interval between courses of treatment varied from 23 to 245 days.

The manner of administering the hormones is indicated in Fig. 1. Data obtained in 52 experiments in which bleeding was precipitated by cessation of treatment (type I, Fig. 1) formed the basis for the present report. In these experiments, no treatment was administered subsequent to the onset of flow.

TABLE 1. EXPERIMENTS OF TYPE I  
No treatment subsequent to onset of flow

Daily Dose		Days Injected	No. of Experiments	Duration of Bleed- ing, Days
Estrogen, I. U.	Progesterone, mg.			
500	1 or 2	28	22	4-50
1250	1	28-39	6	3-18
1250	$\frac{1}{4}$	42-44	7	6-18
500		27-32	5	7-12
1250		28-76	13	7-18

As reported previously (Phelps, 1946b (6)), the cyclic changes in the endometrial vascular bed (Daron, 1936 (3)) were produced in ovariectomized monkeys by injecting ovarian hormones in the manner illustrated in Fig. 1—the changes characteristic of the ovulatory cycle when both estrogen and progesterone were injected, those characteristic of the anovulatory cycle when only estrogen was injected. As observed in intraocular endometrial transplants, the estrogen phase of the experimental cycle produced by the administration of both hormones was characterized by progressive growth of arteries and capillary bed, the progesterone phase by marked arterial development, with increased coiling and tortuosity, formation of anastomoses and a sudden spurt of growth that brought the arteries to the endometrial surface at the stage of the experiment corresponding to the time of nidation. When only estrogen was administered, arterial growth

continued throughout the cycle but the marked vascular development and approximation of arteries and surface characteristic of the response to estrogen-progesterone did not occur.

The pattern of the vascular responses to estrogen-progesterone and estrogen, respectively, was constant. Certain quantitative aspects varied, e.g., extent of vascular growth, tortuosity of arteries, number of arteries activated and duration of bleeding. The variations in the duration of bleeding were noted not only in groups of experiments in which the hormone

### DURATION OF UTERINE BLEEDING

DOSAGE: 500 IU ESTROGEN DAILY, 28 DAYS;  
 ... 1 OR 2 MGM PROGESTERONE DAILY, DAYS 15 - 28.  
 DURATION OF BLEEDING: 4 TO 50 DAYS.

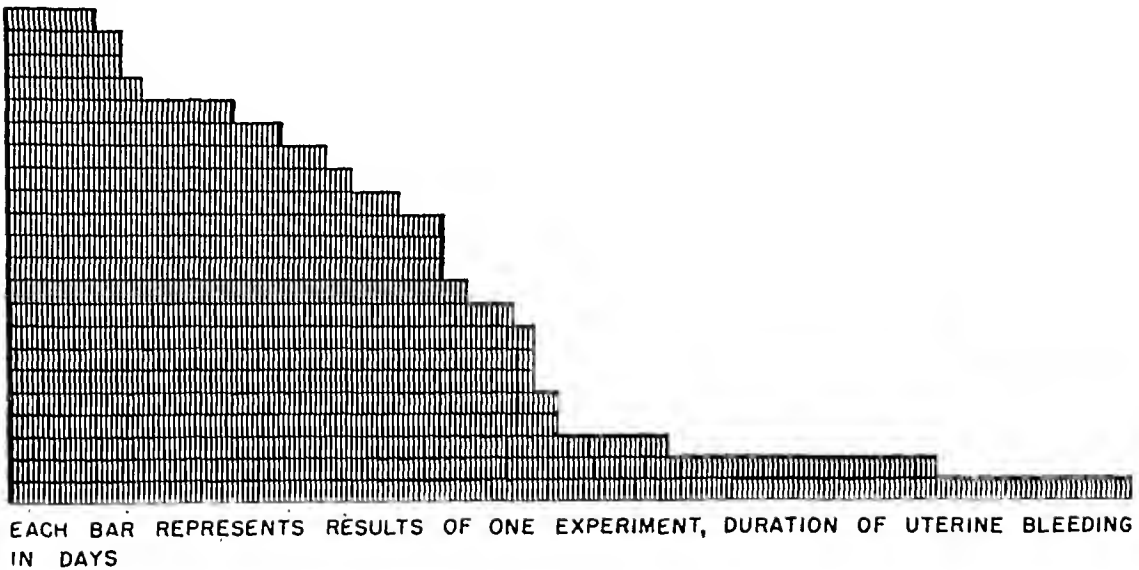


FIG. 2. Duration of the 22 episodes of uterine bleeding under discussion herein, without reference to treatment administered in previous experiments.

dosages employed were different but also in groups of experiments in which the dosages employed were the same (Table 1).

For example, a dosage of 500 international units (0.0166 mg.) of estrogen daily for 28 days, 1 or 2 mg. progesterone daily days 15-28 was administered in 22 experiments. The 22 episodes of uterine bleeding produced varied in duration from 4 to 50 days (Fig. 2.). No significant difference in the results obtained with 1 and 2 mg. of progesterone was apparent. The variations in the duration of bleeding could be correlated roughly with variations in the preexisting vascular structure and with variations in experimental treatment to which the animals had been subjected prior to administration of the dosage in question. By preexisting vascular structure is meant the

specific architecture of the vascular bed of the intraocular endometrial transplants at the beginning of any given course of injections.

An analysis of the 22 episodes of bleeding referred to above in relation to representative variables of the two types described is sufficient for present purposes. Analysis of the results obtained with each of the dosages employed in the study revealed correlations of similar import.

Results obtained in monkey #5 (Table 2) illustrate the relationships observed between the duration of bleeding and the preexisting vascular structure.

TABLE 2. RESULTS OBTAINED IN MONKEY #5; ALL EXPERIMENTS TYPE I

Experiment	Daily Dose		Duration of Injection Period, Days	Duration of Uterine Bleeding, Days*	
	Estrogen, I. U.	Progest- terone, mg.			
1	1250	—	41	8	
2	500	2	28		22
3	1250	$\frac{1}{4}$	43	7	
4	500	2	28		41
5	500	2	28		29
6	500	—	28	8	
7	500	—	28	12	
8	500	—	28	7	
9	500	2	28		12
10	1250	—	28	8	
11	500	2	28		5
12	1250	$\frac{1}{4}$	42	9	
13	500	2	28		19

\* Results obtained with the dosage discussed in the text are shown in the outer column

At the end of experiment 1, the endometrial transplant in the left eye of this monkey was a thin, flat sheet of tissue hardly distinguishable from the iris. A few scattered vessels could be seen in it. It was attached to the iris near the pupil border in the 12 o'clock position and from this point extended upward toward the one o'clock position at the scleral margin.

Comparatively little growth took place in experiment 2. At the end of this experiment, the transplant still appeared as a thin, flat sheet of tissue with few vessels. However, it was a little larger in area than at the beginning of this experiment and extended downward across the upper part of the pupil.

Remarkable growth of the endometrial tissue and vascular bed took

place during experiment 3. At the height of its development in this experiment, the transplant covered about one-fourth of the iris. Two large arteries showed considerable growth during the estrogen phase and continued vascular development during the progesterone phase resulted in the production of an extensive superficial vascular network. Very little tissue was lost during the bleeding phase of the experiment. At the end of the bleeding phase, the transplant appeared as a fairly large and very well vascularized bulbous protrusion. From the under side of the bulbous portion, a thin, flat film extended downward covering most of the pupil. Thus the size of the transplant and the extent of its vascular bed were much greater at the beginning of experiment 4 than at the beginning of experiment 2. The duration of bleeding in experiment 4 was 41 days, as compared with 22 days in experiment 2.

The volume of the transplant at the end of experiment 4 was about the same as at the beginning of this experiment. At the end of experiment 5, the transplant was slightly smaller. It still had a well-developed vascular bed. However, the superficial network was somewhat less extensive and the pattern was different, as compared with conditions at the beginning of this experiment. In one circumscribed area, tissue formed as a result of the growth in experiment 3 became cystic in experiment 4 and was desquamated in experiment 5.

During experiments 6, 7 and 8, in which only estrogen was given, the superficial portion of the extensive vascular bed built up in experiment 3 and maintained in experiments 4 and 5 was destroyed, partly as a result of desquamation during the bleeding phases of these experiments and partly as a result of reversion of some of the arterial branches to a state of relative inactivity. The duration of the bleeding in experiment 9 was only 12 days.

During experiment 6, arterial twigs grew out from the margin of the iris below the bulbous portion of the transplant into the film covering the pupil. These new branches underwent some development during experiments 7 and 8 but at the end of each of these three experiments they became ischemic and, with the exception of a few tiny twigs, did not again become functional until the next course of treatment was started. During experiments 9 and 11 these branches underwent considerable development and bleeding took place from them.

Growth of the endometrial tissue and vascular bed during experiment 12 was greater than during experiment 10 but not as extensive as in experiment 3. The bleeding in experiment 13 was 19 days, as compared with 5 days in experiment 11 and 41 days in experiment 4.

The history of this monkey (#5) indicates that a single course of treatment with ovarian hormones may produce permanent as well as transitory

changes in the structure of the endometrial vascular bed. By transitory is meant changes that are lost during the period of regression and bleeding that follows cessation of treatment. By permanent is meant changes that are not lost during the period of regression. The permanent changes may represent an increase in the total extent of the vascular bed or they may represent a decrease. Extensive portions of the vascular bed built up in one cycle may be destroyed in subsequent cycles. The preexisting vascu-

TABLE 3. DURATION OF UTERINE BLEEDING IN RELATION TO TREATMENT ADMINISTERED IN PRECEDING EXPERIMENT

Monkey	Preceding Experiment			Current Experiment*	
	Daily Dose		Duration of Injection Period, Days	Number	Duration of Uterine Bleeding, Days
	Estrogen, I U.	Progesterone, mg.			
1	1250	—	76	5	6
	1250	—	50	12	5
	500	—	32	7	19
	500	1	28	8	23
2	1250	—	43	2	23
	25000	1	50	4	50
	500	2	28	5	24
	500	2	28	6	24
5	1250	—	41	2	22
	1250	—	28	11	5
	500	—	28	9	12
	1250	$\frac{1}{4}$	43	4	41
	1250	$\frac{1}{4}$	42	13	19
	500	2	28	5	29

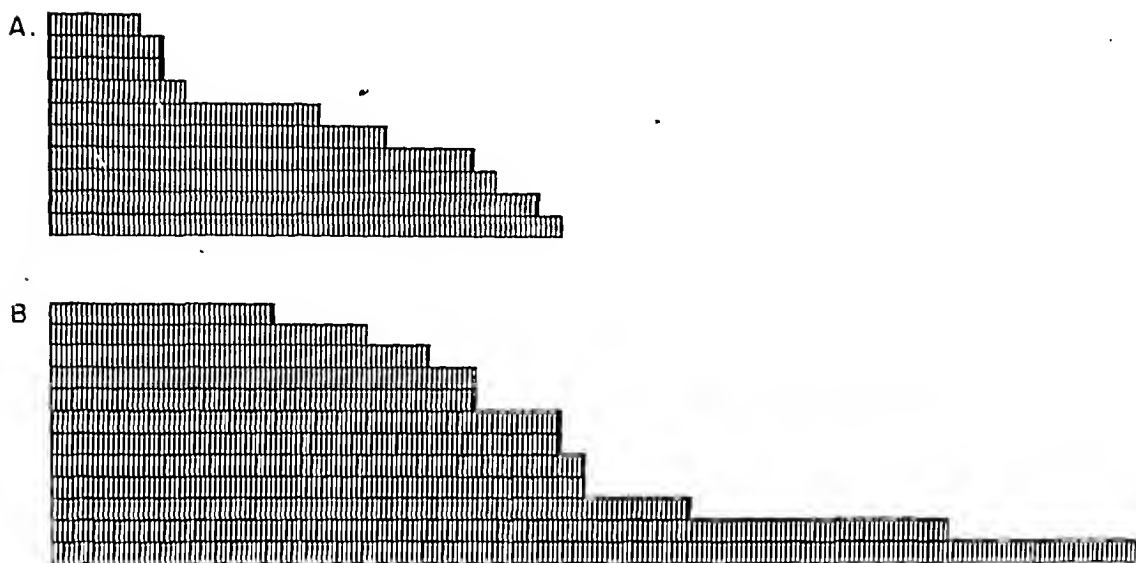
\* Dosage: 500 I.U. estrogen daily, 28 days, 1 or 2 mg. progesterone daily, days 15-28

lar structure may exert a profound influence upon the duration of any single episode of bleeding.

Comparison of the duration of bleeding in experiments 2 and 4 and 11 and 13 indicates that the stimulus acting in the preceding cycle has an important influence upon the duration of the bleeding in the current cycle. Comparison of the results of experiments 1-4 with those of experiments 10-13 indicates that the stimulus acting during the immediately preceding cycle is not the only factor in the previous history that influences the current bleeding. An influence of several previous cycles of stimulation may be evident.



### DURATION OF UTERINE BLEEDING IN RELATION TO HORMONES ADMINISTERED IN PRECEDING EXPERIMENT



HORMONES ADMINISTERED IN PRECEDING EXPERIMENT:  
A ESTROGEN    B ESTROGEN-PROGESTERONE

FIG. 3. Duration of uterine bleeding in relation to hormones administered in the preceding experiment. The bars represent the 22 episodes of bleeding under discussion herein. Each bar indicates the duration of one episode in days. A. Estrogen administered in preceding experiment, duration of 'current' bleeding 4-23 days. B. Estrogen-progesterone administered in preceding experiment, duration of 'current' bleeding 10-50 days.

TABLE 4. DURATION OF UTERINE BLEEDING IN CURRENT EXPERIMENT IN  
RELATION TO HORMONES ADMINISTERED IN PRECEDING EXPERIMENT

Preceding Experiment		Current Experiment		
Hormones Administered	Duration of Injection Period, Days	No. of Experiments	Duration of Uterine Bleeding, Days Range	Average
Estrogen	27-32	4	5-20	14.0
Estrogen-Progesterone	28	6	10-29	22.1
Estrogen	41-43	3	15-23	20.0
Estrogen-Progesterone	42-46	4	14-41	22.5
Estrogen	50-76	3	4-6	5.0
Estrogen-Progesterone	50, 148	2	50, 19	34.5
Estrogen	27-76	10	4-23	13.1
Estrogen-Progesterone	28-148	12	10-50	24.4

The correlations observed between the duration of bleeding and variations in the treatment administered in the experiment immediately preceding afford evidence concerning which aspects of previous treatment may influence the duration of the current<sup>2</sup> bleeding.

The results obtained in monkeys #5, #1 and #2 (Table 3) are particularly significant, since these animals each received 4 or more courses of treatment with the dosage under discussion. In the case of monkey #5 a relation-

#### DURATION OF UTERINE BLEEDING IN RELATION TO DURATION OF TREATMENT IN PRECEDING EXPERIMENT

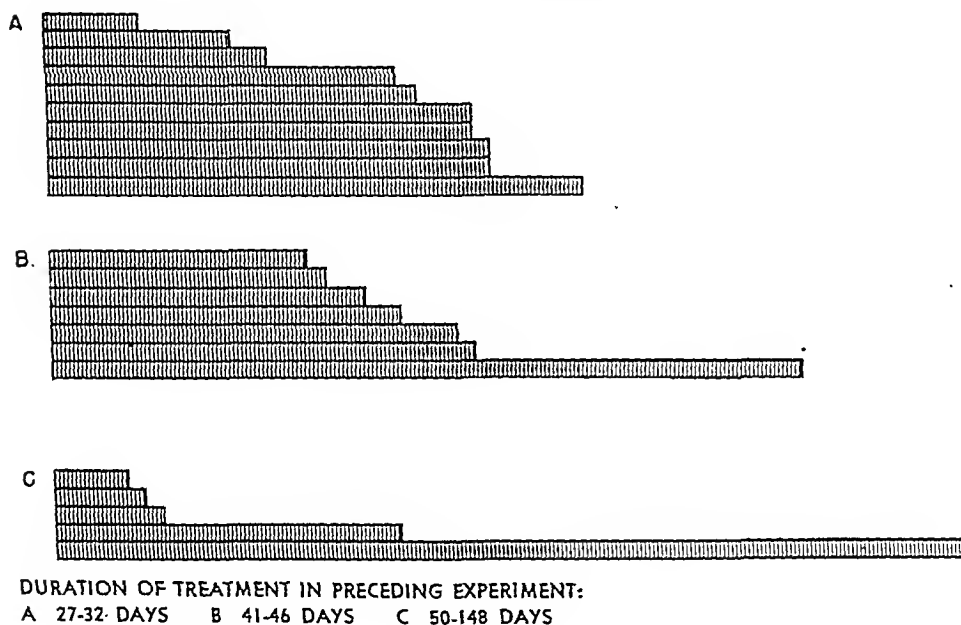


FIG. 4. Duration of uterine bleeding in relation to duration of the preceding experiment. The bars represent the 22 episodes of bleeding under discussion herein. Each bar indicates the duration of one episode in days. A. Duration of preceding experiment 27-32 days, duration of 'current' bleeding 5-29 days. B. Duration of preceding experiment 41-46 days, duration of 'current' bleeding 14-41 days. C. Duration of preceding experiment 50-148 days, duration of 'current' bleeding 4-50 days.

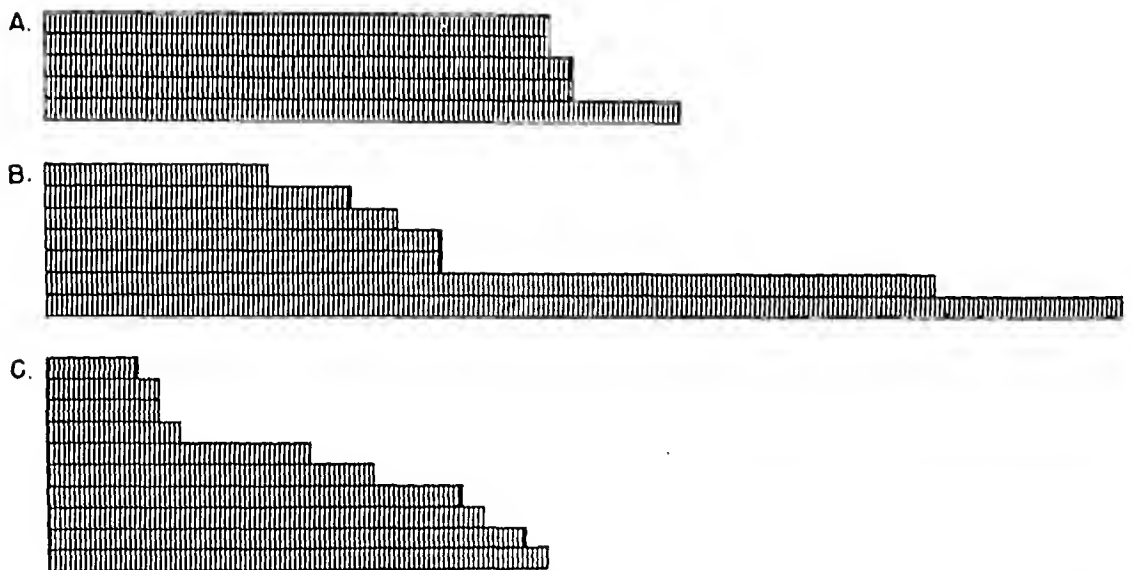
ship between the duration of the current bleeding and the hormones administered in the preceding experiment was apparent, in monkey #1, between the duration of the current bleeding and the duration of the pre-

<sup>2</sup> In the discussion to follow, the word 'current' is used to designate the experiments in which the dosage under discussion was administered.

ceding experiment. Certain variations in the duration of episodes produced in monkeys #2 and #5 could be correlated with variations in the estrogen-progesterone balance in the preceding experiment.

The correlations observed in the group as a whole between the duration of the current bleeding and the hormones administered in the preceding

#### DURATION OF UTERINE BLEEDING IN RELATION TO HORMONES ADMINISTERED AND DAILY DOSAGE IN PRECEDING EXPERIMENT



- A ESTROGEN-PROGESTERONE ADMINISTERED IN PRECEDING EXPERIMENT, DAILY DOSE SAME AS IN CURRENT EXPERIMENT.
- B ESTROGEN-PROGESTERONE ADMINISTERED IN PRECEDING EXPERIMENT, DAILY DOSE NOT SAME AS IN CURRENT EXPERIMENT.
- C ESTROGEN ONLY ADMINISTERED IN PRECEDING EXPERIMENT

FIG. 5. Duration of uterine bleeding in relation to hormones administered and daily dosage in preceding experiment. The bars represent the 22 episodes of bleeding under discussion herein. Each bar indicates the duration of one episode in days. The episodes in question were produced by the administration of 500 i.u. estrogen daily for 28 days, 1 or 2 mg. progesterone daily days 15-28 ('current' experiment). A. Estrogen-progesterone administered in preceding experiment, dosage same as in 'current' experiment; duration of 'current' bleeding 23-29 days. B. Estrogen-progesterone administered in preceding experiment, dosage not same as in 'current' experiment, duration of 'current' bleeding 10-50 days. C. Estrogen administered in preceding experiment, duration of 'current' bleeding 4-23 days.

experiment are shown in Fig. 3. Comparison of the results obtained subsequent to estrogen and estrogen-progesterone administration, respectively, revealed a tendency toward greater prolongation of bleeding in those instances in which both hormones were administered in the preceding experiment. This tendency was still apparent when the factor of variation in the duration of the preceding experiment was eliminated (Table 4).

Fig. 4 shows the duration of the current bleeding in relation to the duration of the preceding experiment. Of interest are 1) the consistent prolongation of bleeding following experiments of 41–46 days' duration (Fig. 4, group B) and 2) the wide range of variation subsequent to prolonged treatment (Fig. 4, group C). An influence of the duration of the preceding experiment was still evident when the factor of variation in the hormones administered was eliminated (cf. Table 5).

The correlations between the duration of the current bleeding and vari-

TABLE 5. DURATION OF UTERINE BLEEDING IN CURRENT EXPERIMENT IN RELATION TO HORMONES ADMINISTERED, DAILY DOSE AND DURATION OF TREATMENT IN PRECEDING EXPERIMENT

Preceding Experiment			Current Experiment*
Daily Dose		Duration of Injection Period, Days	Duration of Uterine Bleeding, Days
Estrogen, I. U.	Progesterone, mg.		
500	1 or 2	28	23, 23, 24, 24, 29
1250	1	28	10
1250	$\frac{1}{4}$	42–44	14, 19, 41
25000	1	46, 50	17, 50
125	$\frac{1}{4}$	148	19
500	—	28, 32	12, 19
1250	—	27, 28	5, 20
1250	—	41–43	15, 22, 23
1250	—	50, 76	5, 6
125	—	51	4

\* Dosage: 500 I.U. estrogen daily, 28 days, 1 or 2 mg. progesterone days 15–28.

ations in the dosages administered in the current and preceding experiments are shown in Fig. 5 and Table 5. Of particular interest are 1) the consistency of the results obtained in the instances in which the hormones administered, daily dosage and duration of treatment were the same in the current and preceding experiments (group A, Fig. 5), 2) the variation in the instances in which one or more of these factors was not the same in the two experiments (groups B and C, Fig. 5) and 3) the range of variation in each of the groups in Fig. 5 as compared with the range in the other two groups.

It seems evident from the data presented in Fig. 3–5 and Table 5 that

uterine bleeding is influenced by each of the following factors: 1) components of the hormonal stimuli acting in previous cycles, 2) duration of previous cycles of stimulation and 3) qualitative and quantitative variation in the stimuli acting in successive cycles. Further study is required to determine the specific influence of each of these factors and the significance of their interaction.

In a previous report (Phelps, 1946a (5)) evidence was presented which indicates that the duration of uterine bleeding is influenced by the components and relative strength of the current hormonal stimulus. The results described herein afford evidence that any given episode of uterine bleeding is also influenced by the components, relative strength and duration of action of hormonal stimuli acting prior to application of the current stimulus. In other words, the influence of a single course of stimulation by ovarian hormones is not limited to the cycle which that course of stimulation represents. Its influence extends through at least one subsequent cycle and probably through more than one. This influence upon subsequent cycles is mediated at least in part through the structural changes produced in the endometrial vascular bed. These changes may be transient or permanent, i.e., carried over into the next cycle. The specific vascular architecture existing at the beginning of any single cycle has an important influence upon the duration of the uterine bleeding in that cycle.

From the practical standpoint, the significance of these findings lies chiefly in the implication that the 'preexisting vascular structure' is one of the factors concerned in the control of uterine bleeding and the demonstration that this factor is subject to alteration as a result of previous hormonal stimulation. Ovarian failure is characterized by variation from time to time in qualitative and quantitative aspects of the endocrine activity of the ovaries. It seems reasonable to assume, therefore, that the preexisting vascular structure, i.e., the structure of the endometrial vascular bed at the beginning of the growth phase of the cycle, is not constant in ovarian failure. In any case, this factor should be taken into consideration in evaluating menstrual symptoms, results of therapy and any other clinical or experimental evidence concerning the action of specific hormones or hormone dosages upon the endometrium.

#### SUMMARY

To obtain information concerning local vascular mechanisms involved in the production of menstrual abnormalities, nine ovariectomized rhesus monkeys were injected with various combinations of the ovarian hormones. Endometrial vascular phenomena were observed by means of intraocular endometrial transplants and these observations were correlated with observations on uterine bleeding. One hundred seven episodes of uterine

bleeding were produced. Analysis of the results of single experiments in relation to treatment administered in previous experiments in the same animal revealed that 1) the influence upon the endometrium of a single course of treatment with ovarian hormones may extend through one or more subsequent cycles, 2) permanent changes in the structure of the endometrial vascular bed may result from stimulation by the ovarian hormones, 3) the architecture of the endometrial vascular bed at the beginning of any given course of treatment with ovarian hormones influences the duration of the uterine bleeding produced by that course of treatment.

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# THE CLINICAL SIGNIFICANCE OF HYPEROSTOSIS FRONTALIS INTERNA

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IN THE 182 years since Morgagni and Santorini (23) first described obesity and virilism associated with the thickening of the internal table of the frontal bone called *Hyperostosis Frontalis Interna* (HFI) a voluminous literature has accumulated dealing with this peculiar osteopathy. With few exceptions (11, 14, 32) great clinical importance has been attached to its presence and the concept that it constitutes a distinct entity has been held by most authors. That the syndrome represents evidence of either endocrine or metabolic pathology has received such universal accord that the diagnosis of *Metabolic Craniopathy* (19, 12) has been incorporated in the recent literature.

The evidence of the existence and distinctiveness of the HFI syndrome has depended largely upon a description of isolated cases or of groups of cases presenting the signs and symptoms considered characteristic of HFI. There has been no attempt in the reported work to compare the clinical features of cases of HFI with the clinical features of a control series of patients without HFI. Because doubt has been expressed in the literature as to the existence of HFI as a clinical entity (14, 32) such a study would be fundamental to a clarification of the subject. We are, therefore, reporting the results of such a comparison of cases of HFI with a control group of patients without HFI; in addition we have reviewed the literature and analyzed our own cases with the purpose of studying the specificity of the HFI syndrome.

The term *Hyperostosis Frontalis Interna* (HFI)<sup>1</sup> refers to a non-inflammatory, frequently symmetrical thickening of the internal table of the frontal bone of the skull. This hyperostosis may or may not extend to adjacent bony areas or to the falx cerebri but it does not involve the external skull surface and thus does not increase the external skull dimensions. Examples of the easily recognized bony thickenings in skull x-rays are shown in Fig. 1. The radiological characteristics have been completely described in Moore's several papers (19, 20, 21).

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<sup>1</sup> Synonyms: Morgagni's syndrome, Stewart-Morel syndrome, Metabolic Craniopathy, Calvarial Hyperostosis, Enostosis of the Calvarium, Exostoses within the Cranium, Intracranial Osteophytes.

The diagnosis depends upon the discovery of HFI associated with any of the following clinical features;—obesity, psychoneurosis and/or psychosis, headache, hirsutism, mental retardation or deterioration, weakness, vertigo, menstrual disorders, tinnitus, hypertension, neurologic disorders, visual disturbances, convulsions, somnolence, lethargy, fatigue, epilepsy,

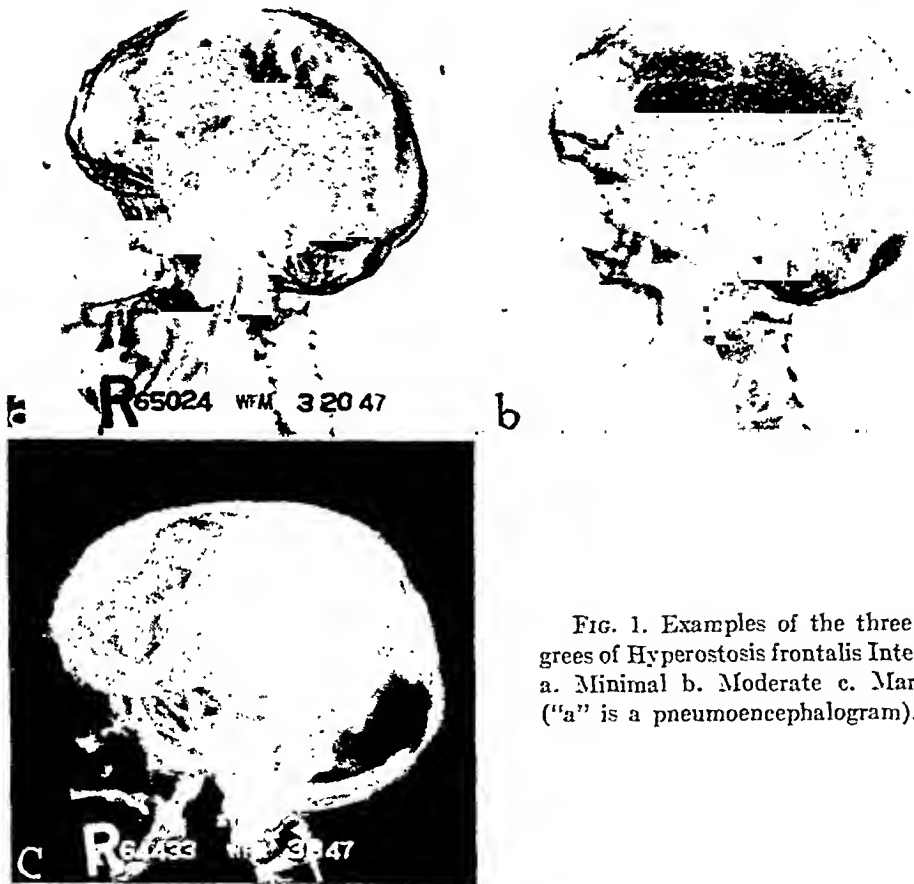


FIG. 1. Examples of the three degrees of Hyperostosis frontalis Interna. a. Minimal b. Moderate c. Marked ("a" is a pneumoencephalogram).

narcolepsy, diabetes mellitus and diabetes insipidus. The first four enumerated might be considered the cardinal features for these are the most commonly reported in the literature.

In 1765 Morgagni and Santorini described the postmortem findings of an obese masculinized 75 year old female who showed numerous intracranial osteophytes of the frontal bone and several bony nodules on the petrous bones. Little clinical significance was ascribed to these changes. In 1928 Grieg (11) recorded his extensive autopsy experiences and exami-



nations of the skulls in the Museum of the Royal College of Surgeons of Edinburgh. He concluded that intracranial osteophytes were very common in old women and were not associated with any particular clinical picture. Most of the literature on the subject, however, stems from Stewart's description (31) in 1928, of 5 insane obese females exhibiting HFI at post-mortem and from Morel's report (22) of a case of obesity, mental disturbances and HFI recognized during life. Since then patients exhibiting HFI with obesity and virilism have been called representative of the Morgagni syndrome; when obesity, mental disturbances and HFI co-exist, the eponym Stewart-Morel syndrome has been used. Knies and LeFever (16), in an exhaustive review of the literature, collected reports of some 300 autopsy and museum specimens and 175 clinical cases from 1900 to 1941.

#### METHODS

The data to be presented have been acquired in the following manner:

a. In order to determine the incidence of HFI in patients who had once required a skull x-ray as part of their case study 150 successive unselected skull films taken in the Mandel Clinic were reviewed. A positive film had to show unequivocal hyperostosis to more than one observer. Doubtful films were excluded. Classification of positive cases into Moore's subgroups was not considered relevant to this investigation.

In order to determine the incidence of HFI in a general hospital and clinic population lateral skull films were taken of 50 unselected females over 30 years of age who had never required a skull film prior to this study.

b. A control group was chosen consisting of 50 female clinic patients, 30 years of age and over, whose clinical picture at one time had required a skull x-ray and this had not shown HFI. Cases were selected whose records were sufficiently detailed to permit an analysis of the significant signs and symptoms.

c. A detailed analysis was made of the clinic and hospital records of 25 patients with HFI similar to the analysis of the control group. Most of these 25 cases were interviewed and examined by the authors.<sup>2</sup>

d. Reports that included substantial numbers of cases were selected from the literature for analysis. Single case reports have not been included. A total of 657 cases was collected. There was no attempt to review the literature completely.

Certain of our criteria in evaluating the importance of signs and symptoms must be elucidated. Cases were classified as showing minimal, moderate or marked HFI on the basis of the degree of the thickening of the

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<sup>2</sup> Doctors Taubenhaus, Bucy (Chicago Memorial Hospital), Allweiss, Sideman, Horner, Spiegel and Boshes permitted us to include their cases.

frontal bone. A representative case of each type is shown in Fig. 1. A patient was considered obese if she was 20 per cent or more above maximum ideal weight (Metropolitan Life Insurance Tables) or if the statement that the patient was obese was repeated several times in the record. "Hirsutism" referred to facial hypertrichosis that was readily evident at a glance and which required removal for cosmetic reasons. A patient was classified as "psychoneurotic" when an important part of his clinical picture was psychogenic in origin. "Hypertension" required a persistent elevation of the diastolic blood pressure to 90 or above.

**Incidence.** The incidence of HFI reported by several authors varies from 0.014 per cent (20) to 40 per cent (14) with an average of approximately 7 per cent. We discovered 4 cases among 150 successive skull films of clinic patients whose clinical status required skull radiograms for various reasons, an incidence of 2.7 per cent. In order to determine the incidence of HFI in a group of women over 30 years of age constituting a general hospital and clinic population, 50 successive women were x-rayed and 6 cases of HFI were discovered, an incidence of 12 per cent.

It is evident that HFI cannot be considered a rare skull anomaly; yet the occurrence of the HFI syndrome has been considered of sufficient moment to warrant the reporting of isolated cases or of small groups of cases in the literature. It appears, therefore, that the occurrence of a specific clinical picture accompanying the calvarial hyperostoses has been merely a fortuitous relationship where it has been reported; the cases not representing the so-called typical picture would have been more frequently revealed in any survey study of sizeable numbers of individuals.

**Sex.** The striking predominance of this craniopathy in females has intrigued all observers. Of 657 cases collected from the literature, 582 were females (88.6 per cent) and only 75 were males (11.4 per cent). If we eliminate from these figures the reports of Carr (6) and Eldridge and Holm (9) who confined their studies to females only, we find the incidence of females to be 84.4 per cent and males 14.6 per cent. Twenty-three of the 25 cases of HFI we studied were females (92 per cent).

**Age.** The greatest incidence of HFI is in middle-aged and elderly females though cases have been reported in teen-age children (16). The average age of cases collected from the literature was 46 years. Among our 25 patients there was an almost even distribution from the third to the seventh decade. The average age was 47.6 years with 11 cases younger than the median and 14 cases older than the median age. The degree of hyperostosis bore no relationship to age, our 6 most severe cases being 27, 31, 35, 49, 55 and 65 years old. Critchley (7) stated that a uniform increase in calvarial thickening occurs in old age which at times forms focal osteophytic processes. He considered HFI a somewhat rare example of these osteophytes.

**Clinical Features.** Eleven papers (23, 11, 14, 32, 19-20-21, 12, 16, 9, 6, 5, 28) were selected from the literature which presented enough detailed data to permit a tabulation of the clinical features considered significant in the HFI syndrome. The incidence of important clinical features in the total of 657 cases from these sources is compared with the 25 cases we observed and with the control group of 50 females without HFI (Table 1). The average ages for the three groups is 46, 47.2 and 47 years. It is evident that there is no significant difference between them. Of the four cardinal

TABLE 1. A COMPARISON OF THE CLINICAL FEATURES OF 657 CASES OF HFI GATHERED FROM THE LITERATURE AND 25 CASES FROM MICHAEL REESE HOSPITAL (MRH) WITH A CONTROL GROUP OF FEMALE CLINIC PATIENTS OVER 30 YEARS OF AGE WITHOUT HFI

Females	Literature	MRH	Control
Average age	89% 46	92% 47.2	100% 47
Obesity (I)	31%	56%	70%
Hirsutism (II)	27	22	10
Headache (III)	29	48	54
Psychoneurosis (IV)	67	44	64
Mental Deterioration or Retardation	35	8	2
Neurologic Disorders	17	8	18
Hypertension	21	40	40
Menstrual Disorders	23	16	40
Tinnitus	21	4	—
Visual Disturbances	15	24	10
Vertigo	25	36	34
Convulsions and Epilepsy	11	4	2
Somnolence	—	4	6
Lethargy	—	0	10
Muscular Weakness	27	44	34
Fatigue	—	56	36
Diabetes Mellitus	4	16	22

features (I, II, III and IV in Table 1) obesity is strikingly more frequent among the controls than in HFI, headache is more frequent in the control group, psychoneurosis is as frequent, and only hirsutism shows a definitely greater incidence among the positive cases. If we add the incidence of the 17 features listed in Table 1 we find in a total of 7 of these features the control group showed the greatest percentage, in 5, the cases collected from the literature predominated, and in 4, our own group of HFI cases presented the greatest frequency of clinical features.

This comparison does not lend itself to a careful statistical evaluation and is open to criticism for several reasons. The groups differ greatly in

total numbers. The evaluations of the 657 cases from the literature were made from eleven different sources with the inevitable differences in criteria, judgment and methods of selection. If we confine our attention, however, to the smaller groups observed in our clinic, the comparison yields the same conclusion. Here again, of the four cardinal features, only hirsutism occurred more often among the HFI cases than among the controls and the incidence of total features occurring with the greater frequency is divided evenly (8 and 8) between the two groups.

Seven of our 25 cases exhibited HFI, obesity and psychoneurosis and would be diagnosed as Stewart-Morel syndrome. We have not encountered an instance of the so-called Morgagni syndrome which comprises HFI with obesity and virilism though 4 of our patients showed HFI, obesity and hirsutism. Two cases exhibited all four of the cardinal features but in 4 cases none of the four existed. Of 6 cases showing only one cardinal feature accompanying HFI, 3 complained of headaches alone, 2 were only obese and 1 was a psychoneurotic. A similar analysis of the control group showed 5 cases with obesity and hirsutism and 22 cases with obesity and psychoneurosis. One control case exhibited the four cardinal features.

The conclusion based on these comparisons becomes inevitable, namely, that the clinical features considered pathognomic of the HFI syndrome can be found as often or oftener among cases without HFI as in those presenting the classical skull changes.

If HFI bears any relationship to the various signs and symptoms to which it has been linked some degree of parallelism should be evident between the severity of the clinical features and the degree of the hyperostosis. Furthermore, progression of the various features should frequently be associated with an increase in the bony thickening. With a single exception (20) neither observation has been made by proponents of the specificity of the syndrome. We classified our cases of HFI as minimal, moderate or marked based on the amount of calvarial thickening and similarly tabulated each clinical feature based on its severity. No relationship between the severity of the hyperostoses and the clinical features was found.

If a syndrome characteristic of HFI exists it should be unusual to find a similar clinical picture occurring in patients without calvarial hyperostoses. In a relatively short time we have observed several such cases and 3 representatives are briefly summarized:

1) Mandel Clinic No. 39036B. A 37 year old white female. Complaints: vague abdominal pain, intermittent amenorrhea since 1934; 30 lb. weight gain in 3 years; occipital headaches, nocturia, "sticking pains" in arms for one year. 1938-Resection of ovarian cyst. 1939-Diabetes mellitus. Phys. Exam: Mentally retarded, moderately obese. B. P. 138/78. Facial hirsutism requiring shaving every other day, abdominal linea striae, left

ovarian cyst. Comment: This case exhibits obesity, hirsutism, headaches, mental retardation, menstrual disorder, diabetes mellitus. The skull x-ray was negative.

2) Mandel Clinic No. 47197B. A 48 year old white female. Complaints: referred to every body system with emphasis on severe headaches, marked fatigue, weakness: previous menorrhagia required hysterectomy. 1940: Diabetes mellitus. Psychiatric opinion: "deepseated masochistic character disturbance with superimposed conversion symptoms." Phys. exam: Marked obesity, extreme nervousness, B.P. 134/80. No hirsutism. Comment: Psychoneurotic patient with headaches, obesity and diabetes mellitus. Skull x-ray was negative.

3) Mandel Clinic No. 89224. A 51 year old white female observed since 1932. Complaints: multitudinous, referred to all body systems but primarily gastro-intestinal and cardiac. Severe headaches, marked weight gain, hot flashes, tinnitus. Phys. exam: Extremely obese, mild hirsutism (plucks facial hair), BP 110/80, varicose veins. Studies: Hypertrophic arthritis of spine, sacro-iliac arthritis, anemia. 1940: Diabetes mellitus. Comment: Psychoneurotic patient with extreme obesity, mild hirsutism, headaches, diabetes mellitus. Skull x-ray was negative.

**Asymptomatic Cases.** Few symptomless cases of HFI have been reported, probably because skull x-rays are usually obtained only in patients presenting complaints referable to the head or indicative of an endocrinopathy and because skull x-ray surveys to determine the incidence of HFI in the general population have rarely been done. When we carried out such a survey study of 50 women 1 symptomless case of HFI was discovered. Grollman and Rousseau (12), who carried out a similar survey, found 2 cases where they considered the skull changes to be merely an incidental finding. Knies and Le Fever (16) discovered HFI in 5 asymptomatic children aged 13 to 19 though 3 of them who were siblings were mentally retarded. The argument has been advanced (12) that symptomless cases will eventually develop the clinical picture of the HFI syndrome because of the progressive nature of the disease. Moore's statement that "in all probability the symptoms antedate the osseous changes" would tend to refute this view. Evidence for progression of the bony changes is scanty (21, 16, 26, 17, 29, 28) with only Eisen and Lehoczky and Orban reporting x-ray studies to confirm this impression. Since most authorities believe that the clinical symptoms and hyperostoses do not parallel each other in severity there is no evidence that the symptomless case, given time, will develop the fullblown HFI syndrome.

**Neuropsychiatric and Mental Disturbances.** The association of psychoneurosis, psychosis, mental retardation and neurologic disturbances with cranial hyperostoses has appeared striking. Studies like those of Eldridge and Holm (9), who found HFI in 50 of 200 successive patients admitted to a mental institution, appear to indicate some distinct relationship between the bony thickenings and the central nervous system. The possibility that the hyperostoses might be responsible for mental aberrations by direct pressure on the frontal lobe of the brain is an attractive hypothesis. Psy-

chiatric patients represent a group most intensively studied for the presence of cranial hyperostoses probably because of the impetus derived from the early reports of Stewart and Morel. In addition skull x-rays are done in many such cases as part of their routine study and for this reason alone many cases have been uncovered. The 25 per cent incidence reported by Eldridge and Holm (9) loses its significance when we find Henschen (14), whose writings indicate an extensive autopsy experience in searching for cranial enostoses, reporting HFI in about 40 per cent of all autopsied females over 40 years of age. As for the possibility of direct cerebral damage caused by the hyperostoses, the evidence militates against such an occurrence. First, because no uniform psychiatric or neurologic picture is associated with HFI (9). Second, because in most instances, signs and symptoms of a neurologic disorder could not be localized to the cerebral region adjacent to the hyperostoses (6, 28). Third, because psychoneurosis and neurologic disorders were found as frequently in the control series of cases without hyperostosis as in patients showing typical frontal bone thickenings and nodulations.

Canavan (5) did find brain atrophy in the frontal region where enostoses occurred in 74 cases but stated that such atrophy was a common change in mental disease. Hemphill and Stengel (13) felt that the progressive dementia and neurologic signs in their case 1 could be explained by brain encroachment but added that the frontal and parietal lobes are most heavily involved in any of the chronic degenerative changes of the cortex and the concept that direct action of the bony condition on brain function is the cause of neurologic or psychiatric changes was not acceptable. Frontal cortical atrophy with HFI has been reported by others (10, 18, 26) but the general opinion appears to be that the atrophy and the hyperostosis are independent manifestations of a generalized process (3).

**Endocrine Nature of HFI.** Reifenstein's recent observation (27) that "obscure diseases usually are not made lucid by incriminating the endocrines," applies to the study of HFI. Here again Stewart's report, which included a description of pituitary changes in 3 of his 5 cases, provided the stimulus for a search for an endocrinopathy and especially for pituitary pathology in cases of HFI. Though the relationship of obesity to anterior pituitary disease has been disproven by the classical investigations of Camus and Roussy (4), Bailey and Bremer (1), P. E. Smith (30) and Hetherington (15), the concept still is popular and is marshalled as evidence for the endocrine etiology of the HFI syndrome. The production of cranial sclerosis in rats by the injection of "purified growth hormone" and "crude pituitary extracts" by Mortimer (24) is frequently quoted as evidence for the pituitary etiology of HFI. Mortimer did not claim to produce cranial hyperostoses, a fact which has not been mentioned in connection with his investigations.

We have observed HFI associated with hyperparathyroidism, hyperthyroidism, acromegaly and the adrenogenital syndrome and the literature contains reports of HFI with myxedema, acromegaly and Cushing's disease. This association of HFI with clearcut endocrinopathies might conceivably be considered evidence for an etiological relationship. The great majority of patients with HFI show no signs of endocrine disease, however, and the very dissimilarity of the diseases listed should dispel ideas of any direct connection. The frequency of hirsutism might indicate some endocrine cause. The etiology of hirsutism remains obscure. Bissell and Williams (2) concluded, from a thorough study of the subject, that the majority of hirsute women showed no demonstrable endocrine disease. They reported that the skull x-rays of their 33 carefully studied patients were negative.

The almost exclusive predilection of any disease for one sex always suggests some sex-linked hormonal cause for the observed anomaly. Yet in the various conditions in which this obtains, such as gout, Buerger's disease and alopecia of the male, no endocrine cause has been established and the predilection of HFI for the female remains unexplained.

**Metabolic Nature of HFI.** The diagnostic designation "metabolic cranio-pathy" has been suggested in lieu of HFI as a more suitable term because of the generalized nature of the disease. The alleged frequency of obesity where HFI is found and the concept that obesity is a metabolic disorder are the principal reasons for the substitution of this vague diagnostic eponym. That obesity is not a metabolic disorder but simply the result of storage as fat of an excess of caloric intake over need has been amply confirmed by many investigators (25).

The probability of a disturbance in calcium metabolism has been accepted by some (6, 20) as the cause of HFI. The localization of the lesions in the skull with absence of other bony pathology and the normal serum calcium and phosphorus studies found in 46 cases (32, 12) should dispel such a concept. Careful studies of calcium balance, however, have not been reported.

Basal metabolic rate and cholesterol determinations, where performed, have revealed no significant abnormalities.

It appears evident that the term "metabolic craniopathy" contributes little to a clearer understanding of the nature of HFI.

**The Hypothalamus and HFI.** With greater appreciation of the vital importance of the hypothalamus in recent years the tendency to link it to various obscure diseases of unknown etiology is to be expected. The autopsy findings of hypothalamic changes in several cases of HFI has suggested an etiological relationship. Reports of cellular changes in the supra-optic nuclei (13), lesions in the floor of the third ventricle (22), and a

questionable solid tumour of the hypothalamic region (20), as well as rare instances of diabetes insipidus (11), are presented in evidence. At the present time the only clinical features that clearly are of hypothalamic origin are diabetes insipidus, temperature and sleep regulation disturbances, experimental obesity in animals, Frohlich's syndrome and experimental manic and rage-like syndromes. In addition, it appears probable that impulses, originating in or transmitted by the hypothalamus, influence the anterior pituitary in causing precocious puberty, ovulation in rabbits, thyroid hypertrophy in response to cold, the alarm reaction (Selye) and possibly hyperthyroidism. It is also known that the hypothalamus has important connections with various cerebral centers. We cannot, in our present state of knowledge, implicate the hypothalamus as the cause of HFI or of most of the protean manifestations that may accompany it.

The numerous theories of the cause of HFI have been amply discussed in the many papers on the subject and since they contribute little to our understanding of this cranial osteopathy, a discussion of them would not be germane to the purpose of this report.

#### COMMENT

This survey of the subject has led us to conclude that no real clinical entity characteristic of HFI exists. The bony thickenings of the internal table of the frontal bone represent a not uncommon incidental finding discovered either by x-ray of the skull or at postmortem and are unrelated to the clinical status of the patient. This conclusion has been based on the evidence presented which is summarized as follows: (1) Though HFI is not a rare finding when skull x-rays of women are carried out, the distinctive clinical picture supposedly linked to it is rare. (2) The clinical features considered pathognomic of the HFI syndrome were found as frequently and sometimes with greater frequency in a control series of women without HFI. (3) There was no parallelism evident between the severity of the clinical features and the degree of the hyperostosis. (4) Many cases have been discovered presenting what are considered the typical features of the HFI syndrome but without the calvarial changes. (5) Symptomless cases with HFI occur. (6) The concept that HFI is closely linked to various psychiatric and neurologic disorders is not valid from the evidence available. (7) There is no evidence to indicate that endocrine, metabolic or hypothalamic pathology causes HFI. (8) The clinical features found in cases of HFI have been so varied that no semblance of unity can be found. A great variety of unrelated disease processes may accompany HFI and can bear no definite relationship to the skull changes.

Our experience with one patient has suggested that the artificial group-



ing of dissimilar clinical features into an entity may elicit certain undesirable sequelae. The various complaints and physical signs displayed by this particular patient had all been ascribed to the presence of a minimal HFI and she had been led to believe that she suffered from an incurable malady. Possibly because of this her psychoneurotic manifestations became deep-rooted and her marked obesity and headaches could not be treated.

### SUMMARY

The question of the existence of a distinct clinical entity accompanying hyperostosis frontalis interna has been investigated. Six hundred and seventy-five cases from the literature and twenty-five of our cases have been analyzed and their clinical features compared to a control series of patients without HFI. The analysis indicates that HFI is a not uncommon incidental skull thickening in women that is unrelated to whatever clinical state may accompany it.

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# ACROMEGALY ASSOCIATED WITH AMYOTROPHIC LATERAL SCLEROSIS AND ACROMEGALY OF THE AMYOTROPHIC TYPE

## REPORT OF 3 CASES

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IT IS always intriguing to study the association of two diseases in which the cause of one is obscure. Under such circumstances ailments previously considered as separate entities have since been found to constitute a single disorder, thereby suggesting the cause and sometimes the cure for the disease. For example, the discovery of pituitary tumor led the way to our knowledge of the source of acromegaly and the development of much information concerning the endocrine control of growth. Generalized osteitis fibrosa cystica was described before it was known that the disease was associated with parathyroid tumors and, through the discovery of their association, hyperparathyroidism was eventually understood.

It is obvious that no causal relationship between acromegaly and amyotrophic lateral sclerosis or muscular dystrophy has been discovered. Nevertheless, we believe that the coexistence of the apparently related changes in the 3 patients reported here as well as similar instances from the literature may represent more than mere coincidence. It may be that the peculiar metabolic imbalance which existed has had a more direct bearing upon the results than can be readily attributed to the disease as we know it. All 3 of our patients suffered from frank acromegaly, and the diagnosis of amyotrophic lateral sclerosis was made in each one by the consultant in neurology. One of the patients died, and the diagnosis of amyotrophic lateral sclerosis was proved at autopsy. We recognized that the subsequent improvement manifested by the remaining 2 patients rendered the diagnosis of amyotrophic lateral sclerosis, as usually accepted, less certain in them, and for that reason these cases might be better classified as the amyotrophic type of acromegaly.

Several similar examples are to be found in the literature. As early as 1891 Duchesneau described the "amyotrophic form of acromegaly," in which he found degeneration of the root fibres in the posterior columns (5). In 1894 Tamburini (11) found in acromegaly a slight degeneration of the root zone in Burdach's column in the cervical and dorsal regions. Schultze (10) described the posterior columns of the cervical region in a patient with

acromegaly as being somewhat lighter with rarefied nerve fibres. Dallemagne (4) presented a patient who had acromegaly and severe central nervous system arteriosclerosis with degeneration in the lateral and posterior columns.

In 1906 Barrett (2) reported the case of a woman with pituitary tumor, acromegaly, and degeneration of the posterior columns of the spinal cord in the cervical and upper dorsal regions. Beduschi (3) in 1907 reported the case of a patient with "the paraplegic form of acromegaly." In 1933 Barker (1) presented a patient with acromegaly and coexistent paresis of the musculature of the lower trunk and thighs.

All physicians who have observed patients with acromegaly are well acquainted with the fact that muscle weakness is a common feature of the condition and that it may become severe. Therefore, it has seemed possible to us that the underlying metabolic strain which is brought to bear upon the tissues in such patients might, if better understood, throw some light on the obscure etiology of some of the muscular dystrophies and perhaps neuropathies.

In man, great interest in the possible relationship of vitamin E to amyotrophic lateral sclerosis followed the work of Einarson and Ringsted (6). For a time there was high hope that a cure might be at hand. Early reports indicated an improvement rate of 20 or 30 per cent (7), but other reports suggested only a short-lived transitory effect (8). An attempt to cover the many clinical reports on the subject is not called for here. In general, it is evident now that the theory that vitamin E can be considered in any sense a cure for amyotrophic lateral sclerosis is untenable in the opinion of most observers. Vitamin E deficiency in many species of animals has been clearly shown to have as its most common manifestation necrosis of striated muscle. Associated lesions in the nervous systems of experimental animals have been reported, but the consensus of opinion seems to be clear that such lesions are not primary, and, according to the careful examination of the problem by Wolf and Pappenheimer (12), such damage is due to some factor other than vitamin E. A splendid review of the experimental evidence, besides those mentioned, is that of Mason (9).

#### CASE REPORTS

*Case 1.* An Italian woman, aged 41, was first seen on July 15, 1933. Her family history was irrelevant. She had had 3 normal children, and her menses, which had begun at the age of 14, had remained normal. She had first noted enlargement of her head and hands following the birth of her last child eight years previously. This condition had become progressively worse during the past few years. Her presenting symptoms included frontal headaches experienced daily for seven years, profuse perspiration, and marked coarsening of the skin. For some years she had complained of constant hunger, increased thirst, and polyuria. In the preceding one and one-half years coarse hair had appeared on

her face. However, the paramount symptom was progressive weakness which had developed in her left arm six months prior to admission and which three months later had spread to involve her legs so that she rose from a chair and walked with great difficulty. For a period of one year there had been a sensation of twitching of the muscles over most of the body (Fig. 1).

On physical examination the blood pressure was 160 systolic and 88 diastolic; the pulse 110. The typical body configuration of acromegaly was evident at a glance. The pupils were equal and reacted, and the fundi were normal. There was no exophthalmos,



FIG. 1. *Case 1.* (a) Patient at age of 29. (b) Patient at age of 41, showing typical features of acromegaly.

nystagmus, or ocular muscle palsies. A large nodular goiter of rubbery consistency was present. The lungs were clear. The heart was rapid but regular, and there was a grade 2 systolic murmur at the base, although there were no signs of cardiac enlargement. The abdominal examination was normal. The neurologic examination revealed marked atrophy of both deltoid muscles as well as the muscles of the arms and forearms. Hoffman's sign was positive bilaterally, and fibrillary twitchings were present in the muscles of both arms. The Babinski sign was questionable bilaterally. The deep tendon reflexes were slightly exaggerated in both arms and both legs. Sensory perception was normal.

X-ray examination of the skull demonstrated an enlarged sella turcica. The visual fields were within normal limits. The basal metabolic rate varied from +35 per cent to +52 per cent. The glucose tolerance test curve was diabetic in type. There was free

hydrochloric acid in the stomach. The urinalysis was normal, and the blood count revealed a slight anemia with a hemoglobin (Haden-Hausser) of 12 Gm. and a leukopenia of 3500 white blood cells. The serum calcium was 11.2, phosphorus 4.4, and urea 33 mg. per 100 cc. The Wassermann and Kahn tests were negative.

At this point, and cognizant of the fact that acromegalics are poor surgical risks, it was decided to subject the patient to pituitary surgery. After iodine preparation a right frontal craniotomy was performed by Dr. W. James Gardner. The patient developed pneumonia and died on the fourth postoperative day. The autopsy revealed a pituitary adenoma of the acidophilic type and degenerative changes in the spinal cord consistent with the diagnosis of amyotrophic lateral sclerosis (Fig. 2).



FIG. 2. Case 1. Dorsal spinal cord showing areas of demyelination in the lateral and anterior funiculi. (Spielmeier's myelin sheath stain)  $\times 10$ . Identical changes were demonstrated in the cervical cord.

*Case 2.* An American woman, aged 66, was first seen on February 2, 1938. The family and past medical history were noncontributory, and a review of the special systems was negative. Her menses had always been normal, with natural menopause at the age of 50. Over the twenty years preceding admission she had noted gradual coarsening and enlargement of her features (Fig. 3). For ten years there had been considerable enlargement of the hands and feet, necessitating an increase in shoe size from  $5\frac{1}{2}$  to 8, and her gloves had to be especially made because of their large size. She had noted increased pigmentation of the skin and excessive perspiration. Approximately one year before admission she experienced soreness in her back following an automobile trip, and five days later her lower extremities became so weak she had difficulty walking. In retrospect she had noted fleeting indications of this difficulty for two or three years. When we saw her the weakness had progressed until she could not walk without assistance and had spread in the intervening few months to involve the muscles of the upper extremities as well. She could not raise her hands to feed herself or comb her hair, and as she lay supine she could not lift her feet from the bed.

Physical examination revealed the typical proportions of an acromegaly. The blood pressure was 160 systolic and 100 diastolic, the pulse rate 98. The skin was thickened, and there was hypertrichosis of mild degree. The skin was unusually dark. She perspired profusely and almost constantly. The pupils were equal, reacted normally, and the fundi, except for some arteriosclerosis of the vessels, were normal. There was no exophthalmos, lid lag, or ocular muscle palsy. No goiter was palpable. The heart, lungs, and abdomen were normal to physical examination. Neurologic examination revealed hyperactivity of



FIG. 3. *Case 2.* (a) Patient at age of 22. (b) Patient at age of 66. Skeletal changes, particularly about joints, apparent. Evidence of interosseous muscle atrophy, especially in left hand. Acromegalic facial characteristics are evident.

all the deep reflexes. Babinski's sign was positive on the left, and the abdominal reflexes were absent. There was marked atrophy of the muscles of the arms and legs, and a few fibrillary twitchings were visible. Sensory perception was normal.

X-ray examination showed ballooning of the sella turcica. Visual fields were within normal limits. The fasting blood sugar ranged from 117 to 152 mg. per 100 cc. Serum calcium was 10, phosphorus 4.1, and cholesterol 130 mg. per 100 cc. of blood. The blood sodium was 365 mg. (normal 330–355), and potassium 20.3 mg. (normal 16–22) per 100 cc. There was slight anemia with hemoglobin 10 Gm. per 100 cc. and eosinophilia 12 per cent. Creatinine and creatine values on twenty-four hour urine specimens were determined on frequent occasions from February 15, 1938, to April 9, 1938. The creatinine varied from 455 mg. to 1180 mg., creatine from 50 mg. to 811 mg. On April 9, 1938, the

twenty-four hour urinary creatinine was 1000 mg. and the creatine was 50 mg. The basal metabolism values were as follows:

2- 8-38	+27	1-31-39	+24
2-10-38	+28	2- 4-39	+39
2-15-38	+15	2 -3-39	+11
2-21-38	+25	3-28-39	+24
2-28-38	+16	7-19-39	+12
4- 7-38	+30	9- 6-40	- 0
4- 8-38	+31		

Muscle biopsies by Dr. B. S. Kline failed to reveal changes sufficient to be of diagnostic significance.

Our diagnosis included adenoma of the anterior lobe of the pituitary (acidophilic) with acromegaly, diabetes mellitus (mild), hypermetabolism with probable hyperthyroidism, and amyotrophic lateral sclerosis.

She was seen by three consultants in neurology. Two agreed to the diagnosis of amyotrophic lateral sclerosis. The third, Dr. S. Baumoe, dissented, pointing out that the course of the disease had not been so relentless as to permit such an unqualified neurologic diagnosis. In the light of subsequent developments his opinion seems to have been justified. The physical findings and the metabolic changes simulate those in *case 1* closely enough, however, to induce us to include the record here for comparison. We have chosen to classify her case as one of acromegaly of the amyotrophic type.

The patient was given vitamin B<sub>1</sub>, 10 mg. three times a day, Lederle's liver extract, 1 cc. daily, Lugol's solution, 1 cc. three times a day for one month, and subsequently fifteen drops three times a day. The dose of liver and Lugol's solution was varied from time to time. Protamine zinc insulin, in doses approximating 10 units daily, as well as a relatively high protein diet was given. From February 21, 1938, until March 3, 1938, the patient was given x-ray therapy to the pituitary and thyroid glands. A total of 1000 r. was given over each temporal area and 1200 r. to the thyroid region.

It is a matter of interest to note that Lugol's solution in doses of 1 cc. three times per day did not result in any depression of the basal metabolic rate over a period of a month, and in smaller doses over a period of many months. This is a rather strong suggestion that factors external to the thyroid were important in the mechanism of increased oxygen consumption. It is also interesting that the radiation therapy administered over the thyroid and pituitary region apparently had no effect upon the basal metabolic rate.

Blood sugar estimations were made both fasting and postprandially on repeated occasions following the beginning of insulin injections and were always found to be normal. Approximately one year later the patient reported that she felt stronger. No fibrillary twitching was seen at that time, and the reflexes were less active. Babinski's sign remained positive on the left. Improvement continued steadily, and in 1940 she was given vitamin E (Ephynal made by Hoffmann-LaRoche) in addition to other medication. In 1946, eight years after we first saw her and at the age of 74, she is able to do her own housework, walk unassisted upstairs, and work in her garden. (A recent examination showed an easily exhausted Babinski sign on the left.)

*Case 3.* A man, aged 52, was first seen on April 22, 1946. He had been aware of an increase of skin coarseness and enlargement of the head and extremities quite typical of acromegaly for the past five or six years. His chief complaint at admission was, however, progressive weakness in both lower extremities for a three-year period prior to this time. He described his legs as "feeling like rubber," and for the three weeks before admission



he had been unable to stand by himself. He noted that his legs were smaller, flabbier, and that the muscles twitched at times. He had no weakness of the upper extremities. There was no headache or visual disturbance. He was irritable and had felt unusually depressed recently, and sexual libido had disappeared. The past history, the family history, and the systemic review were noncontributory. He had 1 child alive and well. Physical examination revealed a blood pressure of 120 systolic and 80 diastolic and a pulse rate of 84. His appearance was typical of severe acromegaly. The pupils were active and equal, and the fundi were normal. The submaxillary, digastric, and anterior cervical glands were slightly enlarged. The thyroid was not palpable. The chest was barrel-shaped, and the lungs were clear. Extreme dorsal kyphosis was present. The heart was normal, and no murmurs were present. The external genitalia were normal. There was marked atrophy and weakness of both legs, most prominent on the right. Fibrillary twitchings were visible in both thighs. Babinski's sign was negative. The deep reflexes were 3+hyperactive except for the right ankle and knee jerk, which were 2+hyperactive. There was no ataxia, and the abdominal reflexes were present over the upper half but absent over the lower half of the abdomen. Photographs were not permitted.

X-ray examination of the skull demonstrated an enlarged sella turcica with smooth contour. The visual fields showed an enlargement of the blind spot on the right eye. The water excretion test (Robinson, Power, Kepler) gave an index of 51.5. Semen examination showed 10,800,000 sperm per cc., a total count of 41,040,000, of which 95 per cent were nonmotile. Urinary gonadotropins measured slightly more than 105.6 mouse units per twenty-four hours. The urinary 17-ketosteroids (ketonic fraction) were 7.3 mg. per twenty-four hours (normal 7-14).

The possibility of deformity of the skull or of the cervical or dorsal regions of the spine as a cause of the neuromuscular changes was seriously considered. Roentgenograms were carefully reviewed with neurosurgeons and radiologists, and those who saw them believed that such an explanation was not valid.

The diagnosis lay in a choice between acromegaly and amyotrophic lateral sclerosis versus acromegaly of the amyotrophic type.

In an attempt to encourage nitrogen retention in the muscles, testosterone propionate 25 mg. every second day and a diet containing 125 Gm. of protein per day were given. One month later the patient reported that he felt stronger generally, though his legs were not definitely improved. Two months later he claimed to be stronger and could walk unsupported, though with much difficulty, and could drive his own automobile, which he had previously been unable to do.

Shortly afterward he died suddenly following a cerebral vascular accident. Autopsy was not obtained.

## SUMMARY

One patient with acromegaly and proved amyotrophic lateral sclerosis is reported. Two patients with acromegaly are reported in whom a clinical diagnosis of amyotrophic lateral sclerosis was strongly suspected. Treatment designed to normalize the metabolic state was followed by slow but pronounced improvement in one. In the other, in a period of less than three months' therapy, suggestive but unconvincing evidence of recovery was seen.

It is suggested that in these patients the increased metabolic demand incident to the acromegaly was an important factor in the production of

the neuromuscular disease which simulated or actually was amyotrophic lateral sclerosis.

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# CONJUNCTIVAL AND CORNEAL LESIONS IN HYPERCALCEMIA\*

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THE hypercalcemia, which accompanies certain disease states and may be the clue to the diagnosis of the underlying pathological physiology, is often overlooked by the clinician. The reasons for this are not far to seek, since the symptoms referable to hypercalcemia per se are usually nondescript and common to many disease patterns—namely, fatigue and vague muscle and bone pains, nausea with or without vomiting, and polyuria (1). In the past few years we have observed, in approximately one-half the patients with hypercalcemia, distinctive phenomena in the eyes which are believed to be the direct result of the hypercalcemia. These observations, together with a brief discussion of the possible mechanism of their production and of their diagnostic value, form the basis of this report.

## DESCRIPTION OF LESIONS

Two distinct types of ocular abnormalities have been observed; one in the conjunctiva, the other in the cornea. Neither can be identified except with the aid of the slit lamp. The conjunctival lesions appear as small glass-like particles (Fig. 1) within the conjunctiva of the palpebral fissure region. These minute areas are crystal clear, and not at all like the grayish patches sometimes seen extending superficially over a pterygium. The lesions may be many or few. Sometimes there is accompanying redness of the conjunctiva with complaint of conjunctival irritation; the latter may even be the patients' most annoying symptom. In two instances biopsies of the lesions have been obtained. The amount of tissue obtainable was, of course, very minute. Microscopic sections were made from one bit of tissue and chemical analysis was performed on the other. The deposits were identified by a member of the Johns Hopkins University Department of Chemistry as "probably calcium phosphate." The patient suffered from hyperparathyroidism. This determination was made six years ago, and we have no way now of finding out what methods were used in identification. Furthermore, the note states that "there were many technical difficulties involved." Microscopic sections taken from the other biopsied specimen,

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which was removed from a patient with vitamin D poisoning, are depicted in Figures 2 and 3. Dr. Richard H. Follis' description of these follows:

"The epithelium in general is normal. Just beneath the basal layer and lying in the most superficial connective tissue, there is purplish staining amorphous material which is more refractile than the rest of the tissue. There is no cellular reaction about this material. With the Von Kossa silver stain this material stains an intense black and is found to be granular and to be present in larger quantity than was suspected with the H and E stain. In most of the sites of deposition of this material, the overlying epithelial cells are entirely normal. However, in several areas, where the



FIG. 1

silver staining substance is greatest, the basal epithelial cells show vacuolization."

The second type of ocular abnormality observed occurs in the cornea. The corneal changes (Fig. 4) consist of hazy grayish granular epithelial and subepithelial opacities running concentrically with the limbus, on either the nasal or temporal side or both. The opacity is most dense at the periphery, fading out centrally, but may faintly involve almost the entire cornea. Its appearance is quite similar to that of band keratitis which is ordinarily seen in association with intraocular inflammation and is familiar to all ophthalmologists. There is, however, a single difference; in the corneal lesion here described there are clear areas within the area of opacity. Band keratitis which is occasionally reported as a primary disorder is extremely rare. "Primary" band keratitis may be progressive and seriously affect vision. Its etiology is unknown and it well may be that some such cases may have been due to hypercalcemia. The corneal involvement associated

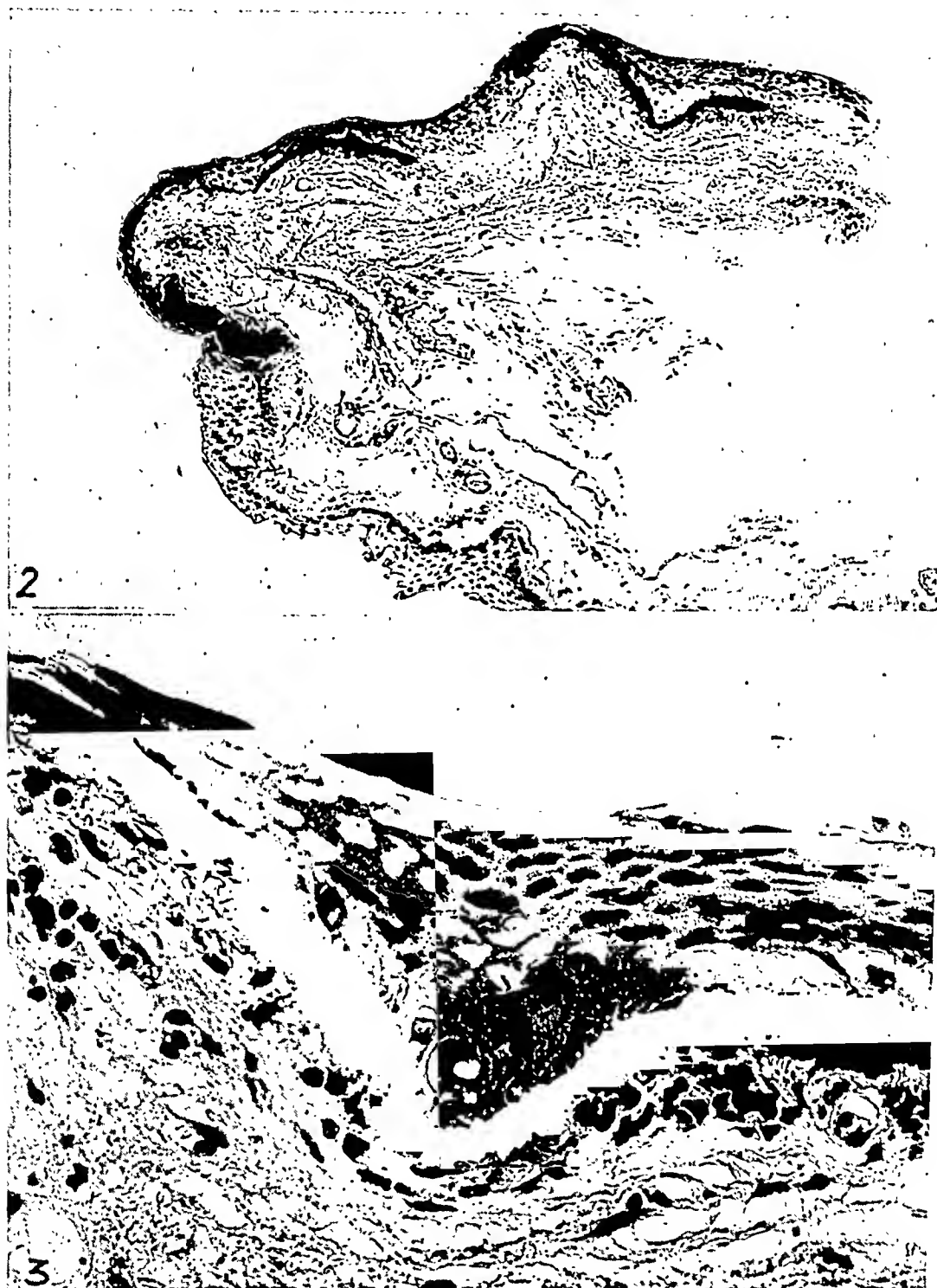


FIG. 2 and 3. *Case #414780* Biopsy of conjunctiva. Tissue immediately placed in neutral formalin (formalin 10% saturated with magnesium carbonate). Pieces were imbedded in paraffin, sectioned and stained with von Kossa technique. Fig. 2.— $\times 100$  magnification. Fig. 3.— $\times 450$  magnification.

with hypercalcemia should not be confused with arcus senilis since the opacities are much more superficial. Also the lesions are easily distinguished from the white limbus girdle described by Vogt.

#### ANALYSIS OF CASES

Over the 10-year period since the first conjunctival lesion of this type was noted, it has been possible for us to examine by slit lamp 20 patients suffering with elevation of the serum calcium. In eight instances calcium depositions in the conjunctiva were found; there were five instances of



FIG. 4

band keratitis in our series, and in all but one there were also present conjunctival lesions. Since 1940 ocular lesions have been looked for in hypercalcemic patients at the Massachusetts General Hospital, and seven instances of the band keratitis lesions have been encountered. As yet no conjunctival lesions have been observed by them. We have Dr. David Cogan's permission to include his findings in this report (2) and the combined cases will be discussed as a whole. Thus there have been seen to date 16 patients with distinctive ocular lesions of these two types in hypercalcemic patients, 12 corneal, eight conjunctival (five having lesions of both types). In hundreds of patients examined by one of us (Walsh) with the slit lamp during the last 10 years, no patient without hypercalcemia has shown conjunctival depositions of the kind described above. Since band

keratitis lesions of the so-called idiopathic type (i.e., without intraocular inflammation) are extremely rare, the occurrence of 12 instances of such lesions in patients with hypercalcemia is striking. It would seem that the ocular lesions in both these types are definitely related to, and probably the result of, hypercalcemia.

#### TYPES OF CASES IN WHICH THE OCULAR MANIFESTATIONS OCCUR

Conjunctival deposits have been seen associated only with hyperparathyroidism and poisoning from vitamin D. Concentration of calcium in the serum of these patients has ranged from 12 to 19 mg. per 100 cc. Cases with vitamin D poisoning all had concentrations of serum inorganic phosphorus of 3.5 mg. per 100 cc. or greater. Only one such case was without evidence of serious renal damage from the drug; the others all manifested albuminuria and casts, elevated non-protein nitrogen in the blood, with impaired ability to form concentrated urine and to excrete phenolsulfonphthalein. The patients with hyperparathyroidism without serious renal impairment had low concentration of inorganic phosphorus in the serum (Table I). In three instances the appearance of the conjunctival deposits has changed markedly after the hypercalcemia has been corrected. One of these, a patient suffering with mild vitamin D poisoning without noteworthy renal complications, showed only a few of the crystalline deposits in the conjunctiva; when observed four months later with normal serum calcium, the deposits had entirely disappeared. A physician with hyperparathyroidism and renal insufficiency had many conjunctival deposits prior to parathyroid surgery; two years later, during which interval mild hypoparathyroidism had been present, all but a few of the conjunctival lesions had disappeared despite progression of renal insufficiency and the development of hypertension.

Another case is worthy of special comment. This patient had for many years suffered with hypocalcemic tetany, probably due to a combination of deficiency of vitamin D further enhanced by hypoparathyroidism after thyroidectomy. She was given 250,000 units of calciferol daily for 15 days. Serum calcium and phosphorus altered with unexpected rapidity from 7.7 mg. and 3.4 mg., respectively, per 100 cc. to 15.8 mg. and 4.3 mg. Routine slit lamp examination had been performed just prior to calciferol administration with negative findings. On the 15th day when signs of vitamin D poisoning became apparent, there were readily visible characteristic conjunctival lesions. The patient left the Johns Hopkins Hospital and was followed thereafter by Dr. Milton F. Little. Two weeks after the lesions had appeared, they were seen to be still present. Five months later reexamination by Dr. Little showed that the lesions had entirely disappeared.

TABLE I

History No. Sex      Age	Diagnosis	Ca mg. %	P mg. %	NPN mg. %	Prot. Gm. %	Ocular Lesions
J.H.H. 168084 F      40	Vitamin D poisoning	13.6	3.9	46.0	7.0	Crystals in bulbar conjunctiva.
J.H.H. 421206 F      62	Vitamin D poisoning	15.1	5.2	75.0	6.6	Conjunctival crystals and band keratitis, bilateral.
J.H.H. 414730 F      59	Vitamin D poisoning	14.9	3.5	57.0	7.0	Conjunctival crystals and band keratitis.
J.H.H. 366421 F      36	Vitamin D poisoning	15.8	4.3	23.0	7.1	Conjunctival crystals appeared within 10 days.
J.H.H. 419594 M      54	Vitamin D poisoning	13.6	4.6	89.0	6.7	Conjunctival crystals and band keratitis, bilateral.
J.H.H. 204618 M      31	Hyperparathyroidism	12.7	7.3	82.0	6.8	Conjunctival crystals and band keratitis, bilateral.
J.H.H. 190342 M      49	Hyperparathyroidism	19.3	4.3	90.0	6.3	Conjunctival crystals.
J.H.H. 194939 M      59	Vitamin D poisoning	14.3	4.6	68.0	7.0	Band keratitis. No conjunctival lesions.
J.H.H. 224565 F      31	Hyperparathyroidism	13.6	1.7	36.0	7.0	Conjunctival crystals.
M.G.H. 324826 M      49	Hyperparathyroidism	12.4	6.6	Elevated	—	Abortive band keratitis. Conjunctival calcification.
M.G.H. 268258 M      25	Hyperparathyroidism	12.3	4.8	Elevated	—	Narrow strip in corneal limbus.
M.G.H. 391430 M      25	Sarcoid ? beryllium poisoning	15.6	3.1	26.0	6.7	Chalk-like opacities of limbal cornea.
M.G.H. 235557 M      44	Hyperparathyroidism	12.0	4.7	132.0	8.6	Band keratitis.
M.G.H. 252484 F      36	Sarcoid	14.4	4.1	75.0	7.0	Abortive band keratitis.
M.G.H. 254784 M      27	Vitamin D poisoning	12.6	4.3	52.0	5.6	Abortive band keratitis.
M.G.H. 589 F      59	Hyperparathyroidism	12.4	2.4	23.0	6.2	Abortive band keratitis.

In the Johns Hopkins series of hypercalcemic cases examined by slit lamp, there have been five proven instances of hyperparathyroidism in which neither type of ocular lesion was manifest. Six patients, who were suffering from multiple myeloma, sarcoid or skeletal carcinomatosis, have been examined; none thus far have manifested either type of ocular lesion, nor have uremic cases with normal serum calcium and high serum phosphorus concentrations. However, Cogan has seen two instances of band keratitis in patients with hypercalcemia in association with sarcoid.



The keratitic lesions have appeared five times in the Johns Hopkins series, in four patients with vitamin D poisoning and renal insufficiency, in one patient with hyperparathyroidism who also had renal insufficiency. When first seen, serum calcium levels in the vitamin D cases were 13.6 mg. or higher per 100 cc., with phosphorus 3.5 mg. or above. The hyperparathyroid patient's serum calcium was 13.2 mg., phosphorus 3.8 mg. (he suffered also with renal insufficiency). Of Cogan's seven cases, only one suffered with vitamin D poisoning, four had hyperparathyroidism (one with low serum phosphorus without renal insufficiency, the other three with elevated non-protein nitrogen and serum phosphorus) and two patients had sarcoid. The serum calcium concentration in this group varied from 12.0 to 15.6 mg. per 100 cc.

### DISCUSSION

We are not, at this time, informed as to the exact nature of the lesions here reported. It has been assumed that the lesions seen in the conjunctiva with the slit lamp as refractile glass-like particles are, in fact, the sub-epithelial basophilic deposits so clearly outlined by the silver stain in the biopsy specimen. The pathological specimen demonstrated that the size of the areas is of the correct order, and the deposits lie just beneath the epithelial layer, both facts which make the assumption more tenable. The clinical lesions have never been seen by us or our colleagues in patients who did not have hypercalcemia, but a study of conjunctival biopsies is now in progress, designed to determine if pathological lesions of this sort occur in normal or other disease states.

It is also entirely an assumption that the material in the lesions is calcium and that the salt is phosphate. The staining reaction is not altogether specific. By all odds the cation most likely to have been present in such a situation, however, is calcium; and the single specimen chemically analyzed lends strong corroboration. The hypercalcemic state (presumably higher than normal ionized and diffusible calcium) is the only factor common to all the patients, so far as we have been able to discern. Certainly the variations in serum concentration of phosphorus in this series were great, from levels of 7.3 mg. per 100 cc. to low levels in the uncomplicated cases of hyperparathyroidism.

We are equally lacking in knowledge as to why the calcium salt, if such it be, is deposited in the conjunctival basement membrane, and whether or not it is a deposit of the same salt beneath the surface epithelium of the cornea which causes the band keratitis. It may be that, since basement membranes in general are basophilic, the ocular lesions are but visible manifestations of deposits in many basement membranes under metabolic conditions similar to the ones met with in these patients (3). Certainly

deposits in the basement membranes of the renal tubules in hypercalcemia and alkalosis have a similar appearance.

We are wont to classify pathological calcification into two groups: 1) In one group are those cases in which it appears likely that a local process, usually degenerative, has produced changes conducive to the deposition of lime salts; in this group are the calcific lesions seen in the fat necrosis after leakage of pancreatic juice, calcification in arteriosclerotic plaques, in muscles after some hemorrhages and myositis ossificans, and the lesions of so-called idiopathic calcinosis. 2) The other group includes disease states which are accompanied by changes in the concentrations of ionized calcium or inorganic phosphorus, or both, in the serum. Examples of this type are vicarious calcification seen in chronic alkalosis, certain cases of renal insufficiency, hyperparathyroidism and vitamin D poisoning.

The ocular lesions herein described would seem clearly to fall within the latter group. There must, however, be some factor other than hypercalcemia, as yet unknown, in the production of these ocular lesions, for they have not been observed in *all* patients with vitamin D poisoning or hyperparathyroidism, and in our small group we have not been able to correlate the degree of elevation of serum calcium nor the duration of the pathological state with their appearance.

It is hoped that further studies will aid in elucidating some of the mechanisms of calcification, still so poorly understood. Recognition of such lesions by ophthalmologists and other clinicians may also serve as an important clue to diagnosis of patients with hypercalcemia, in whom the fundamental pathological physiology might not have been suspected otherwise.

#### SUMMARY

Ocular lesions of two types, identifiable only by slit lamp examination, have been observed in patients with hypercalcemia. In one type there are small glass-like particles in the conjunctiva of the palpebral fissure area. Biopsy of one of these lesions showed a deposit of amorphous material, presumably a calcium salt, beneath the conjunctival basement membrane. Eight examples of this ocular abnormality are reported among 20 patients with hypercalcemia examined.

Associated corneal lesions have been observed in four of these cases, and an additional seven have been seen by Cogan and his associates at the Massachusetts General Hospital. The corneal changes consist of hazy grayish granular epithelial and subepithelial deposits running concentrically with the limbus on either the nasal or temporal side or both. These are quite similar to band keratitis, a picture familiar to ophthalmologists.

The only metabolic abnormality common to the entire group with these two types of ocular lesions has been hypercalcemia. The lesions have been

observed in hyperparathyroidism, vitamin D poisoning and twice in patients with sarcoid. The lesions have been associated with both very high and very low concentrations of serum phosphorus. In two instances the conjunctival lesions have disappeared after the hypercalcemia was corrected. So far the corneal lesions have not been observed to disappear.

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# PREGNANCY TEST USING THE MALE TOAD

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**I**N THIS report a new pregnancy test is described, in which the male toad (*Bufo arenarum* Hensel) is used as the reacting animal.

The positivity or negativity of the test is determined by the presence or absence of spermatozoa in the urine of the toad previously injected with the urine of a woman suspected to be pregnant.

The spermatozoa of the adult toad are in contact with the Sertoli cells and are grouped together, parallel to each other, in such a way that they appear like bundles. Occasionally the spermatozoa are free and detached from the wall of the tubes and can be seen as a loose skein.

The relation between the pituitary and the testis of *Bufo arenarum* Hensel has been studied by Houssay and Lascano Gonzalez (2). These authors found evidence that in the toad the pituitary exerts a constant influence upon the spermatogenetic activity of the testis, and that removal of the pituitary produces testicular atrophy. Repeated subcutaneous implantation of toad pituitary caused testicular hypertrophy in normals and pituitarectomized animals.

The administration of toad pituitary to the toad produces the detachment of the spermatozoa, which appear free in the interior of the tubes. The phenomenon of the detachment of the spermatozoa and their migration through the kidney has been well studied by De Robertis, Burgos and Breyter (3) in the *Bufo arenarum* Hensel. Following their detachment the spermatozoa migrate to the bladder and are excreted with the urine.

Based on the results shown in this report, the pregnant woman's urine also has the property to induce the detachment and migration of the spermatozoa, which, some time after the injection, can be found in the urine contained in the bladder.

The results here presented are comparable to those obtained in the rabbit, because in every case, simultaneously with the reaction in the toad, a Friedman test was performed using the same pregnant woman's urine.

## METHODS

**Test animals:** Toads weighing more than 100 grams were used. Toads of less than 100 grams are apt to have incomplete spermatogenesis. The

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majority of toads were recently captured. Others had been in captivity more than one month. In some reactions, toads injected one week before were used again.

**Urine injected:** 10 cubic centimeters of the first morning specimen of urine from the suspected pregnant woman was injected into the lateral lymphatic sac of each toad. The urine was not subjected to any special previous preparation (filtration, pH, concentration, etc.). All the injections were made during the morning. From that time on the toads were kept in their basins or cages. No special illumination was used, the toads being kept under the natural light of the laboratory. Simultaneously, a Friedman test was performed with urine taken from the same sample, for the purpose of determining the coincidence of the two tests. The Friedman reactions were carried out independently by other investigators.

**Procurement of urine samples from the toad:** The urine was obtained from the toad by introducing a pipette into the anal canal. The pipettes used are of variable gauge, approximately the same as the ones of one cubic centimeter. To collect the urine the toad is dorsally held on the table, the pipette is introduced no deeper than 0.5 or 1 cm. in the anal canal and is gently moved back and forward until a drop of urine is seen to penetrate the pipette. It is of importance, when the pipette is introduced into the canal, that the end of the pipette be held upwards. This position of the pipette facilitates the collection of the urine. No more than a drop is necessary. By extracting this small amount the bladder is not emptied, and, if necessary, subsequent samples can be collected.

**Microscopic observation:** A drop of the urine thus obtained is placed on a slide and observed directly under the microscope without previous smearing, fixing or staining. A strong illumination is not necessary. The spermatozoa are seen in great quantities as delicate undulating short dark lines.

**Time of observation:** Most of the toads' urines are examined 1, 2, 3 and 22 hours after the injection of woman's urine. In order to determine how long a positive reaction shows spermatozoa in the toad urine, some observations were made until 3 days after the injection.

**Season of the year:** These experiments were performed during the months of January and February, 1947, that is to say, during the summer months.

## RESULTS

Total number of reactions.....	102
Deaths due to the injection { toad.....	1
{ rabbit.....	2
Comparable results.....	99
Consistent results.....	94

Inconsistent results	{ toad positive—rabbit negative.....	3	
	{ rabbit positive—toad negative.....	2	
Positive results.....			62
Positive results observed at 1, 2, 3 hours after injection.....			47
Positives after 1 hour.....		9	
Positives after 2 hours.....		36	
Positives after 3 hours.....		2	
		<hr/>	
	Total.....	47	
Positive results observed during 3 days.....			18
Maximum time that spermatozoa could be found in the urine of these 18 toads after injection.....			50 hours

**Control experiments:** As a control of this reaction, the urine of 77 different subjects and several substances were injected. The 77 urines tested gave uniformly negative results. The urines injected were obtained from:

Children: 5 males, 10 females.

Normal adults with active sexual life: 18 men, 20 women.

Women at menopause: With symptomatology 6; without symptomatology 5.

Men above 60: 4.

Thyrototoxic adult women: 4.

Secondary amenorrhea: 3.

Hirsutism: 2.

**Other substances:** Other substances were injected as controls. The following enumeration shows the name of the substance injected along with the dose per toad, the number of toads used and the results obtained. In every case the dose was given in one injection and observations were made within 3 hours and at 24 hours after the injection.

Estrogens: 10,000 u. estradiol benzoate.....	12 toads—negative
1 mg. stilbestrol.....	14 toads—negative
Progesterone: 5 mg.....	5 toads—negative
Testosterone propionate: 10 r. g.....	6 toads—negative
Thyroxin: 1 mg.....	5 toads—negative
Insulin: 2 units.....	6 toads—negative
Adrenaline: 0.5 cc. (1:1000).....	10 toads—negative
Desoxyeorticosterone Acetate 10 mg.....	6 toads—negative
Chorionic Gonadotropin: 1–10 u.....	5 toads—negative
100 u.....	3 toads..... Positive 1 hour
500–1000–1500 u.....	8 toads..... Positive 30 minutes
Serum Gonadotropin: 500 u.....	12 toads..... 4 Positive 2 hours
	..... 4 Positive 22 hours
	..... 4 Negative 48 hours

**Temperature:** The influence of this factor was studied by injecting 500 u. of chorionic gonadotropin per toad, into 12 toads kept for 22 hours at different temperatures. The rectal temperature of each toad was meas-

ured at the end of the 22 hour period, when the gonadotropin was injected. Of the toads with an average temperature of 26° C., 3 were positive 30 minutes after the injection and all at one hour. Of the toads with an average temperature of 16° C., only one was positive at 30 minutes, and all at one hour. Of the toads with an average temperature of 9° C., no one was positive at 30 minutes, 4 were positive at one hour, and all one hour and 45 minutes after the injection.

## DISCUSSION

Based on the results reported, it seems that the urine of pregnant women has the property of producing the detachment and migration of the toad's spermatozoa. The negative results, obtained with the control substances injected—with the exception of chorionic and serum gonadotropins—and the consistency of results with the ones obtained when performing the Friedman tests, suggest that the substance responsible for the reaction is the gonadotropin produced by the placenta.

Under the conditions in which these experiments were performed (season of the year, temperature, etc.) it can be concluded that this pregnancy test has the following advantages:

1. **Speed of the reaction:** All 47 positive reactions observed at 1, 2 and 3 hours were positive within 3 hours (9 in one hour, 36 in two, and the remaining 2 in three hours). The reactions which were negative at three hours remained so at 24 hours.

2. **Simplicity:**

- (a) No special preparation of the urine to be injected is needed.
- (b) The injection is without difficulties.
- (c) The collection of the toad's urine is simple and no surgical intervention is needed.
- (d) The microscopic observation is immediate, without previous histological procedures.
- (e) The microscopic differentiation of spermatozoa is simple because of their number and well-defined characteristics of morphology and movement.
- (f) The several steps of the technique can be performed rapidly. Usually one observation will not take more than one minute from the moment that the toad is taken from the cage until the result is established.

3. **Clear end-point:** The positivity of the reaction is clearly indicated by the presence of spermatozoa in the toad's urine.

4. **Specificity:** Based on the results obtained with the control urines, this reaction is specific for pregnant women's urine.

5. **Economy:** The economy of the procedure is due not only to the low cost of the toad, but also because no special care is needed, i.e., food, handling, etc. In addition, the same toad can be used again after an interval of one week between reactions. The real practical value of this reaction is not definitely established by the data presented in this paper because of the

small number of urine examinations and because it might happen that the reaction of the toad could be different during other seasons of the year, i.e., during the mating period or variations of temperature. It would be of general interest to know if this reaction for the *Bufo arenarum* Hensel is also true for other species.

The species difference in this type of gonadotropic reaction is clearly demonstrated by the impossibility of producing ovulation in the female *Bufo arenarum* Hensel by the injection of pregnant woman's urine, a fact clearly established for the *Xenopus Laevis* by Shapiro and Zwarenstein in 1934 (4).

### CONCLUSIONS

1. The injection of 10 cubic centimeters of pregnant woman's urine is followed by the appearance of spermatozoa in the urine of the toad *Bufo arenarum* Hensel.

2. Of 99 pregnancy tests simultaneously performed in the toad and in the rabbit, 94 results were consistent. Of the remaining 5, 3 toads were positive, and 2 rabbits were positive.

3. In 47 positive reactions observed, spermatozoa appeared in the toad's urine within 3 hours after the injection. The negative results during this time were still negative after 24 hours.

4. In a group of 18 toads, once the spermatozoa appeared in the urine, it was possible to observe spermatozoa for a maximum of 50 hours.

5. Seventy-seven urines from non-pregnant subjects gave negative results.

6. The individual injection of certain doses of estrogens, progesterone, testosterone, thyroxin, insulin, adrenaline, and desoxycorticosterone were without effect.

7. The injection of individual doses of 100, 500, 1000 and 1500 I.U. of chorionic gonadotropin gave positive results, and the injection of 500 I.U. of serum gonadotropin gave some positive results.

8. Temperature seems to be a factor of importance in the speed of the reaction.

9. Under the conditions in which these experiments were performed (season of year, temperature, etc.) the results obtained are stimulating and could be the base for a new pregnancy test. The characteristics of this test can be summarized as follows:

- A. Speed of the reaction
- B. Simplicity
- C. Specificity
- D. Clear end-point
- E. Economy



## SUMMARY

A new pregnancy test is described in which the male toad (*Bufo arenarum* Hensel) is used as the test animal.

The injection of urine from a pregnant woman has the property of producing the migration of the spermatozoa from the testicles of the toad to the bladder. The spermatozoa can be observed under the microscope in a drop of toad's urine obtained by catheterization.

Ninety-nine comparative results with the Friedman reaction simultaneously made, and the results of 86 control experiments are shown.

Under the conditions in which this study was made, this pregnancy test has the advantages of speed of the reaction, simplicity, specificity, clear end-point, and economy.

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# Letter to the Editor

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TO THE EDITOR:

## AN OBSERVATION ON MENSTRUAL MISBEHAVIORS

**T**HE normal functions or balance of the glands of internal secretion, or the endocrine system, are subject to extraneous influences.

Shortly after returning to private practice from military service, one of the observations that struck us most forcibly was the large number of menstrual misbehaviors, either irregular cycles or dribbling or excessive flow in many of the younger patients seen. The probability of there having occurred during the war years sufficient stress, strain, anxiety, and emotional disturbance to produce a glandular imbalance seemed not unlikely.

With this in mind, and with the observation of an apparent preponderance of menstrual disturbances in younger individuals, it was felt that a study comparing a pre-war year with the last year of war might reveal some difference in the incidence of menstrual disorders. For this purpose, records were chosen for the years 1940 and 1945. Those individuals within the age groups from 19 to 39 only were considered. The only menstrual disorders recorded in the study were those diagnosed as menorrhagia, metrorrhagia, and dysmenorrhea. The findings were borne out by pathological reports of scrapings. If there was any question of pregnancy, such cases were eliminated. Likewise, fibroid growths and other pathological processes of the pelvis were eliminated as far as possible. In other words, only the actual functional disturbance of menstruation was recorded in this study.

Figures were obtained from two of the metropolitan hospitals in the city of Dallas for the pre-war year 1940, at which time there was a total of 82 cases of menstrual misbehavior of the type mentioned between the ages of 19 and 39, and a total of all-age admissions of females of 9,141. In 1945, which represents the cumulative effect of "war nerves," there was a total of 368 menstrual disorders amongst a population of all-age admissions of 12,398. These figures were tested for their significance. The difference in proportions of the two years was taken as indicated by the mathematics shown at the top of the next page.

The standard error of the difference was thus computed, which was found to be .0019. The difference in proportion divided by the standard error of the difference, which is considered a formal significance test, is 11 times the standard error of difference. Biometrically, any difference that is greater than twice the standard error is considered significant; that is, it is considered not to be a result of chance observation.

TABLE I. BASIC DATA

	Total Admissions		Functional Menstrual Disorders	
1940	9,141		82	
1945	12,398		358	
Biometric Analysis				
	+	-	Total	Proportion
1940	82	9,059	9,141	.0090
1945	368	12,030	12,398	.0297
Total	450	21,089	21,539	.0209

Difference in proportion  $+ = .0297 - .0090 = .0207$

Standard Error of Difference  $= \sqrt{\frac{(.0209)(.8791)}{9141} + \frac{(.0209)(.8791)}{12398}}$

$= \sqrt{\frac{.018373}{9141} + \frac{.018373}{12398}}$

$= \sqrt{.00000201 + .00000148}$

$= \sqrt{.00000349}$

$= .0019$

Difference	.0207
Standard Error of Difference	.0019
$= \frac{.0207}{.0019} = 11$	

The observation, therefore, leads one to feel that the four years of war tension played a rather striking role in traumatizing the glandular balance resulting in an endocrine imbalance as judged by the increased incidence of menorrhagia, metrorrhagia, and dysmenorrhea. We are certainly cognizant of other possible variables that doubtless contributed to this finding, but feel that the most important factor or factors were the results of the anxiety state that was admittedly present among the daughters, wives, or sweethearts of the men who were serving in the Armed Forces, to say nothing of the general and over-all effect on the population and its social fabrications.

It is hoped that this observation will be duplicated in some other hospitals in order to see if the same results are noted. Such knowledge adds

immeasurably, should it prove to be true, to our concept and increasing knowledge of psychosomatic medicine or functional disturbances in general. Its implications, further, as to the possible care and therapeusis for such conditions, both in individual peacetime care of the patient or in the presence of another holocaust would likewise be informative.

J. SHIRLEY SWEENEY

A. E. HALEY AND LEONA YAKLIN

Fort Logan Veterans' Hospital

Denver, Colorado

April 28, 1947

The senior author (J.S.S.) wishes to acknowledge with thanks the help of Lowell J. Reed, Vice President of Johns Hopkins University, in making the biometric analysis included in this study.

## Book Review

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RIDDLE, OSCAR AND ASSOCIATES. *Studies on Carbohydrate and Fat Metabolism. With Especial Reference to the Pigeon.* Washington, D. C. Carnegie Institution of Washington (Pub. 569).

This volume provides information on hitherto little-known aspects of the physiology of the pigeon, and places in relief the relatively few observed differences in mechanisms for the regulation of carbohydrate and fat metabolism in birds and mammals. The various investigations deal especially with the role of the several hypophyseal hormones in the regulation of blood sugar, ketosis, muscle and liver glycogen, heat production, plasma fat, and liver fat. Tests with hypophyseal hormones were supplemented by tests with insulin, thyroxin, sex steroids, and hormones of the adrenal cortex. Rabbits and rats, in addition to pigeons, were used in some of these studies. The action (and assay) of pituitary extracts obtained by fractionation with ammonium sulfate was studied extensively, and the results fully related to those of previous investigators in the field. Supplementing the use of intact pigeons, several hundreds of tests were made on birds from which one or another hormone-producing gland was removed—namely, thyroids, pancreas, one or both lobes of the pituitary, testes, ovaries, adrenals, and parathyroids. Cyclic glycemias and lipemias associated with egg production in pigeons and doves were found; and some evidence is reported which supports the view that hormones of the adrenal cortex and ovary, respectively, induce this cyclic fluctuation of these two substances in the blood.



for work done. The amount of the fellowship is \$2,500.00 annually. The nominee must possess the degree of Doctor of Philosophy or Doctor of Medicine or their equivalent. It is suggested that no restriction be placed on age, but that preference be given to applicants who have recently completed the requirements for their Ph. D. or M. D. degree. The nominee must present evidence of scientific ability as attested by studies completed or in progress and/or the recommendation of responsible individuals; submit a program of proposed study; indicate one or more institutions where the proposed program will be carried out; submit statement of approval from the investigators with whom he proposes to conduct his research; serve full time if awarded a fellowship. A small amount of time (10 to 15 per cent) may be allotted for course work or for participation in teaching, the latter purely on a voluntary basis.

## AMERICAN ASSOCIATION FOR THE STUDY OF GOITER

### VAN METER PRIZE AWARD

The American Association for the Study of Goiter again offers the Van Meter Prize Award of Three Hundred Dollars and two honorable mentions for the best essays submitted concerning original work on problems related to the thyroid gland. The Award will be made at the annual meeting of the Association which will be held in Toronto, Canada, May 6th, 7th, 8th, 1948 providing essays of sufficient merit are presented in competition.

The competing essays may cover either clinical or research investigations; should not exceed three thousand words in length; must be presented in English; and a typewritten double spaced copy sent to the corresponding secretary, Dr. T. C. Davison, 207 Doctors Building, Atlanta 3, Georgia not later than February 1st, 1948. The committee, who will review the manuscripts, is composed of men well qualified to judge the merits of the competing essays.

A place will be reserved on the program of the annual meeting for presentation of the Prize Award Essay by the author if it is possible for him to attend. The essay will be published in the annual Proceedings of the Association. This will not prevent its further publication, however, in any Journal selected by the author.

T. C. DAVISON,  
*Corresponding Secretary*

# The Journal of CLINICAL ENDOCRINOLOGY

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## THE SYNDROME OF CONGENITALLY APLASTIC OVARIES WITH SEXUAL INFANTILISM, HIGH URINARY GONADOTROPINS, SHORT STATURE AND OTHER CONGENITAL ABNORMALITIES

TABULAR PRESENTATION OF TWENTY-FIVE PREVIOUSLY  
UNPUBLISHED CASES\*

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**I**N 1938, Turner (7) reported 10 instances of a new syndrome, confined to females, and characterized by sexual infantilism, short stature, webbing of the neck, and cubitus valgus. This was the first report in the American literature calling attention to the syndrome, which later was found to include congenitally aplastic ovaries. The next contribution to the understanding of this condition appeared in 1942 when Varney, Kenyon, and Koch (8) described 4 short, sexually retarded females, all of whom excreted abnormally large amounts of urinary gonadotropins; indeed their titers equalled those of castrate or postmenopausal women. On the basis of 7

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\* Presented in abbreviated form at the 28th Annual Meeting of the Association for the Study of Internal Secretions, San Francisco, June 29, 1946. Since this paper was sent to press, one of the patients (Case 5) was included in an article by one of us (Minnie B. Goldberg) with Maxwell and Smith as co-authors, which appeared in this Journal, Vol. 7, p. 11, January, 1947.

scattered autopsy reports discovered in the foreign literature, they suggested the importance of ovarian aplasia as one of the cardinal characteristics of the syndrome. Later that same year, Albright, Smith, and Fraser (1) submitted 11 patients of their own, particularly stressing the primary ovarian insufficiency and discussing its differentiation from primary pituitary infantilism. Two of their patients had been examined by means of the peritoneoscope and no ovaries were seen. In 1943, Schneider and McCullagh (5) described 5 cases of "Turner's syndrome." In 1944, Shereshevski (6) reported 6 cases, the characteristics of which seemed to justify their inclusion in this group. One of these cases had been published previously in 1925, but even in 1944 the author was unaware of the articles referred to above and made no mention of congenitally absent ovaries or high urinary gonadotropins. A review of the literature with tabular presentation of most of the previously reported cases was published in 1944 by Wilkins and Fleischmann (9). Their article included also a table of 18 cases in which autopsy or exploratory laparotomy revealed absent or rudimentary ovaries. These authors added 5 cases of their own, 4 of them confirmed by biopsy studies.

"Ovarian agenesis" is the name that Wilkins and Fleischmann selected for this syndrome. We have refrained from employing this title, since in our opinion, it attaches undue and unwarranted significance to this feature, and seems to imply that the other characteristics such as short stature and various congenital abnormalities are consequent to the ovarian aplasia. Such an assumption seems to us most unlikely as it did to the above authors. This will be discussed later in this paper.

As presently accepted, the syndrome is characterized by congenitally aplastic or completely absent ovaries, sexual infantilism, high urinary gonadotropins, and usually by short stature. Other occasional findings are webbed neck, cubitus valgus, coarctation of the aorta, and other congenital abnormalities. To date about 60 cases have been published. We are adding 25 patients, the diagnosis in 6 having been proven by exploratory laparotomy or peritonoscopy. Of the remainder, 10 patients were found to have an abnormally high urinary gonadotropin (FSH) titer. In the other 9 patients, the diagnosis is presumptive, based on typical clinical characteristics. To conserve space, the customary detailed presentation of each case is omitted, all pertinent data being compressed into tabular form.

#### DISCUSSION OF DIAGNOSTIC CRITERIA IN TABLE I

These patients came under our observation at ages varying from 8 to 39 years. It is to be noted, however, that the diagnosis of ovarian aplasia was not entertained in some instances until several years after they were first seen.

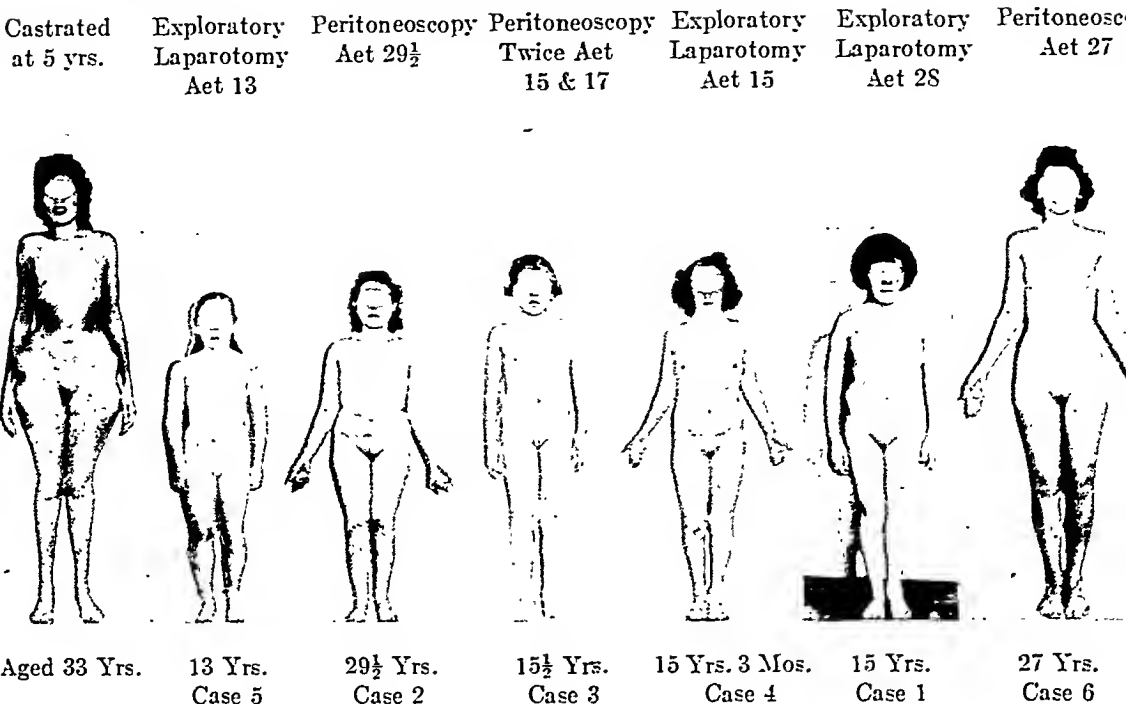


FIG. 1. Proven Ovarian Aplasia

Six cases of ovarian aplasia, 3 proven by exploratory laparotomy and 3 by peritoneoscopy; and for contrast a 33 year old female who was castrated at 5 years of age by two ovariectomies, six months apart, the first because of an ovarian hematoma and the second because of an ovarian cyst with twisted pedicle. She shows the typical disproportionately long extremities and trochanteric fat pads characteristic of so-called preadolescent eunuchism and eunuchoidism. Five of the 6 congenital cases are under 55 inches tall. The sixth is the only exceptionally tall patient in the entire series of 25 cases. See Figure 4 for appearance of pelvis in *case 4*.

It may at once seem surprising that this syndrome should receive consideration in a girl only 13 years of age (the youngest case on record in which the diagnosis has been verified (*case 5*, Figure 1), since the menarche normally often does not occur until 14 to 16 years of age. This girl had been studied for five years previously because of failure to grow. The stunting in association with sexual infantilism aroused the suspicion of ovarian aplasia. Sixteen of 25 patients (64 per cent) were reported as being "always small"; of the remaining 9, the onset of stunting was noted between the ages of 4 and 13. In this group, therefore, short stature was apparent in the majority during the first year of life, and at the latest by the age of 13 years.<sup>1</sup>

<sup>1</sup> In only 1 of our patients (*case 8*) was there any suggestion of the association of injury with the onset of stunting.



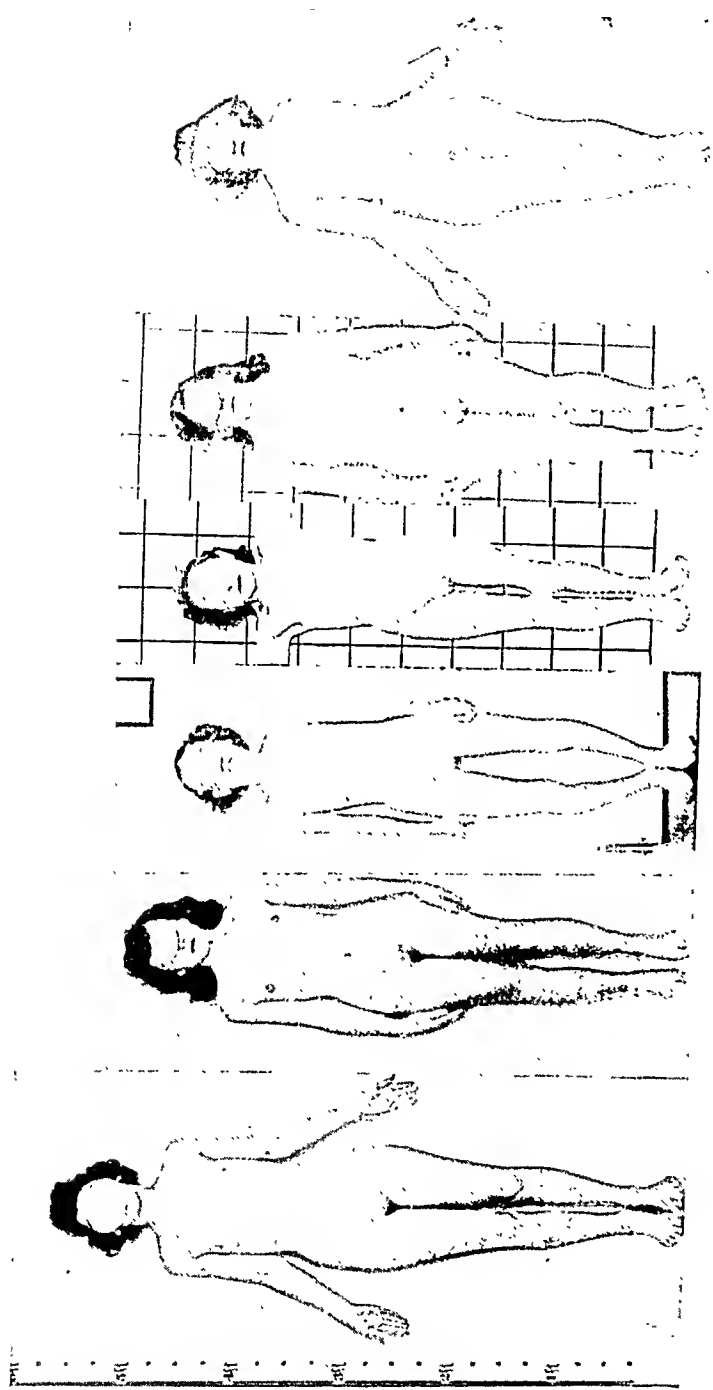
No.	Patient	Age	Onset of Stunting	Height	Build	"Shield-like" Chest	Breasts	Sexual Hair		External, Internal Genitalia
								Pubic	Axillary	
13.	K.M.—private patient, Dr. M. Goldberg	18 (1946)	Always small	59½ in. (150.5 cm.)	Boyish	0	Slight development. Had had previous treatment.	Moderate. Previous treatment.	Moderate	Infantile
14.	C.A.—U-51073	16 (1946)	Always small	47½ in. (121 cm.)	Medium	0	0	Very sparse	Very sparse	Small. Uterus & ovaries not felt
15.	H.W.—U-132055	31 (1946)	Always short	54 in. (137 cm.)	Slightly stocky	Slightly+	Fair development	Normal	Normal	Small cervix & uterus. Ovaries not felt
16.	L.B.—U-121978	22 (1945)	13	54 in. (137 cm.)	Stocky	0	Well developed pubertal type, small nipples	Sparse	Sparse	Small uterus. Ovaries not felt

## PRESUMPTIVE CASES

7.	A.C.—OPD-114588	24 (1924)		56½ in. (143 cm.)	Thin	+	0	Sparse	Sparse	Infantile. Ovaries not felt
8.	M.S.—U-72366	15 (1929)	Always small	54½ in. (138.5 cm.)	Stocky	+	0	Sparse	0	Infantile
9.	B.H.—private patient, Dr. H. Lissner	30 (1932)	Always short	58½ in. (149.5 cm.)	Medium	+	0	Sparse	Sparse	Very small uterus. Ovaries not felt
10.	L.W.—U-47669	18 (1939)	Under 13	58½ in. (148 cm.)	Slightly stocky	+	0	Sparse	Sparse	Infantile. Tiny cervix only
11.	H.W.—U-74112	22 (1941)	4-5	53½ in. (138 cm.)			0	0	0	Infantile. Ovaries not felt
12.	R.D.—private patient, Dr. H. Lissner	21 (1942)	Always short	56½ in. (143 cm.)	Stocky with large legs	+	Underdeveloped	Sparse	Sparse	Infantile, endoscopic shows cervix less than 1 cm. in diameter
13.	D.M.—private patient, Dr. H. Lissner	15 (1942)	Always short	53½ in. (136 cm.)	Slightly stocky	+	Underdeveloped	Sparse	0	Infantile. Ovaries not felt
14.	E.H.—private patient, Dr. H. Lissner	30 (1943)		61½ in. (156 cm.)	Stocky	+	0	Sparse	Moderate	Infantile. Ovaries not felt
15.	K.D.—private patient, Dr. H. Lissner	16 (1943)	Always short	56½ in. (143 cm.)	Stocky	+	0	Sparse	0	Ovaries not felt

Menstruation	Blood Pressure	Short or Webbed Neck	Cubitus Valgus	Other Congenital Abnormalities	Urinary Gonadotropin Excretion	Bone Age	Osteoporosis	Exploratory Laparotomy or Peritoneoscopy	Additional Data
0	96/40	0	0	0	+192 m.u. -238 m.u.	18	0	0	
0	100/40	+	0	Congenital heart disease.	+253 m.u. -334 m.u.	14	0	0	Looked young
0	132/70	0	+	Rudimentary 1st rib on left. Fusion of 1st & 2nd ribs on rt.	+184 m.u. -303 m.u.	between 21-25	+	0	Has slight tendency to hirsutism, moderate progeria
One scant period aet. 17	115/72	+			+334 m.u. -480 m.u.	Adult	0	0	Married "happily" five years

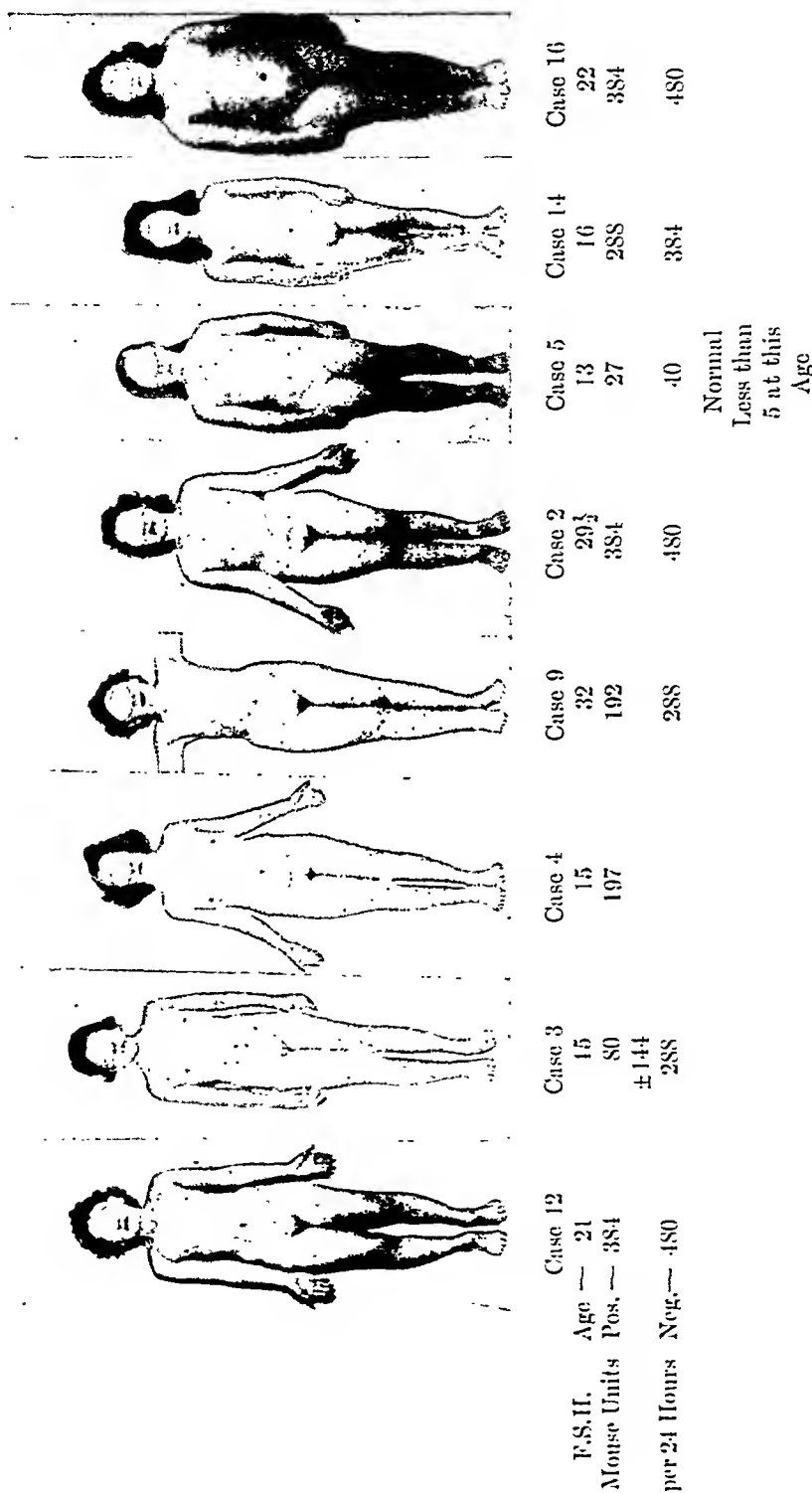
0	140/100	+		Blowing systolic heart murmur.		19-20		0	Looked young. Occasional hot flashes. Mental age 13. BMR +3%
0		+		Excess glial tissue in eyes. Spina bifida occulta		15-16	0	0	
0	110/60	+				At least 22 yrs.	0	0	
0	128/80	0		0		Retarded about 3 yrs.	0	0	Hot flashes from age 15
0	160/100			Coarctation of the aorta. Double left kidney. pelvis, & ureter			+	0	Facelooked old, body looked young. Had severe rheumatoid arthritis. Sella "rather small." BMR +9 and +33%. Cholesterol 175
0	158/90	+	+	Madelung's deformity of wrists		21		0	Looked young. Was a teacher. Chest x-ray showed small heart & aorta, but no evidence of coarctation
0	106/50	0	+			14		0	Looked act. 12-13. Bluish sclerae. Deep voice
0	110/75	+	+	Madelung's deformity of wrist joint		Adult		0	Nails fragile, skin and hair dry, slow mental reactions, alopecia areata. BMR -5%
1 scant period 4 mos. before	104/76	+	+			14-15		0	Low pitched voice



	Case 6	Case 13	Case 7	Case 10	Case 11	Case 15
F.S.H.	Age — 27	18	21	39	27	31
Mouse Units	Pos. — 96	192	96	288	165	184
per 24 Hours	Neg.—192	288	192	384	240	308

FIG. 2. Cases with High Urinary Gonadotropins

Patients with abnormally high FSH titers. Of these 5 were verified and are seen also in Figure 1 (cases 2, 3, 4, 5, and 6). The patient on the extreme right differs from the rest in having a history of one scant menstrual period at 17 years of age (see discussion in text under breast development). Note eubitus valgus (exaggerated carrying angle) in cases 2, 4, 6, and 15, and compare with normal appearance of arms in case 12. The short webbed neck is clearly noticeable in cases 4, 9, and 11.



Another Characteristic Case (S) (No Photos) Positive at 288  
Negative at 384

FIG. 2 (continued). Cases with high urinary gonadotropins

In this series the height ranged from  $47\frac{1}{2}$  inches (120.5 cm.) to  $67\frac{1}{2}$  inches (171 cm.) with an average of 55.2 inches (140 cm.). It is to be noted, however, that only 5 of the 25 patients exceeded 57 inches (145 cm.), and their heights were, respectively,  $58\frac{1}{2}$  inches (148 cm.),  $58\frac{7}{8}$  inches (149.5 cm.),  $59\frac{1}{2}$  inches (150.5 cm.),  $61\frac{1}{4}$  inches (156 cm.), and  $67\frac{1}{2}$  inches (171 cm.). Interestingly enough, peritoneoscopy in the exceptionally tall patient (*case 6*, Figure 1) revealed an empty pelvis. From these figures it is reasonable to conclude that patients with congenital ovarian aplasia as a rule are under 57 inches (145 cm.). It should be noted, however, that in the literature reviewed and cases reported by Wilkins and Fleischmann, (9) 6 out of 47 cases were found with a height of over 60 inches, and 4 of these were over 62 inches. The tallest was the patient of Pela (3) who was reported to be 70 inches in height; at autopsy the ovaries were described as the "size of orange seeds."

Nevertheless, we do not believe that the ovarian aplasia bears any etiologic relationship to the short stature exhibited by the great majority of these cases. We oppose the concept of sexogenous dwarfism and are disposed to explain the deficient growth on a genetic rather than an endocrine basis.

Sixteen of the 25 patients (64 per cent) were described as having a stocky build. Only 2 were considered thin. A characteristic "shield shaped chest" was another frequent finding. The thorax was prominent anteriorly, broader than normal with widely spaced nipples, and with an increased antero posterior diameter similar to the "barrel chest" of emphysema. This "shield chest", which has not been emphasized heretofore, was noted in 17 of our 25 cases (68 per cent) (especially marked in *cases 1, 2, 4, 11, 18, 22, and 25*. See Figures 1, 2, and 3). The chest enlargement showed a remarkable correlation with stocky build, the association occurring in all but 3 cases. It may emphasize the clinical impression of stockiness.

As would be expected in this condition characterized by sexual retardation, breast development was completely absent in 20 of the 25 cases. Slight development existed in 4 previous to any estrogen therapy. An exception was in the well-developed breasts of a rather atypical patient (*case 16*, Figure 2) who had had a single spontaneous menstrual period at 17 years of age, five years before she came for examination. Curiously enough, her level of gonadotropin excretion was next to the highest of those tested (384 mouse units per twenty-four hours).

A very sparse amount of pubic hair was present in most of these cases. Axillary hair, if present at all, was very sparse indeed. *Case 15* constitutes the single exception. This patient exhibited a constitutional hirsutism, which however, was not heterosexual. It will be noted in her photograph (Figure 2) that her pubic hair was abundant and the axillary hair was also

normal in amount. She had had considerable estrogenic therapy prior to the photograph but claimed that neither the sexual hair nor the slight breast development was influenced thereby. This will be discussed further under therapy.

The external genitalia were invariably infantile, but in 3 instances (*cases 4, 10, and 16*) the patients were married and claimed satisfactory sexual relations. A third married patient (*case 9*) also reported satisfactory marital relations, but her vagina was found to be so small that normal coitus seemed most improbable. In 1 patient (*case 6*) a plastic operation had been necessary to form even a small vagina. *Case 15* admitted having had intercourse on several occasions, and from the pelvic examination this seemed probable.

As regards the internal genitalia, no adnexa could be felt in any of the patients by rectal or pelvic palpation. In 8 instances the uterus or cervix were described as very tiny.

Primary amenorrhea existed in all but 2 of the 25 patients, these 2 each having had a single scant period before receiving any endocrine therapy. One of these (*case 25*, Figure 3) showed the stigmata of Turner's syndrome, with webbed neck and cubitus valgus. The other patient already referred to (*case 16*) had rather well developed breasts and high urinary gonadotropin excretion. Unfortunately neither of these patients was explored, but the evidence suggests that some ovarian tissue was probably present and that a high urinary gonadotropin titer does not necessarily indicate complete absence of gonadal tissue.

In 5 cases the systolic blood pressure was under 100 mm. of Hg, the lowest level being 88. We do not attach any significance to these mildly low levels. On the contrary, it should be noted that in 4 instances the systolic pressure was 140 or over—namely, 140, 158, 160, and 200 mm. Hg. Coarctation of the aorta was proved in only 1 of these. Wilkins and Fleischmann (9) likewise noted hypertension in some of their cases.

Of the stigmata emphasized by Turner, (7) webbing or apparent shortening of the neck was noted in 14 of our 25 cases (56 per cent). Cubitus valgus was specifically looked for in 16 cases. It was present in 9 and suggestive in 1 (total—62 per cent of cases tested) (See Figure 2).

Eleven of the 25 cases showed other congenital abnormalities. Four of these involved the cardiovascular system; coarctation occurred only once. Of particular interest were the findings in *case 1* (Figure 1) where the patient was explored in an effort to find the cause for repeated severe gastrointestinal hemorrhages. It was noted by the surgeon<sup>2</sup> that there were no arterial or venous arcades in the mesentery of the small bowel and the

<sup>2</sup> Dr. Leo Eloesser of San Francisco.

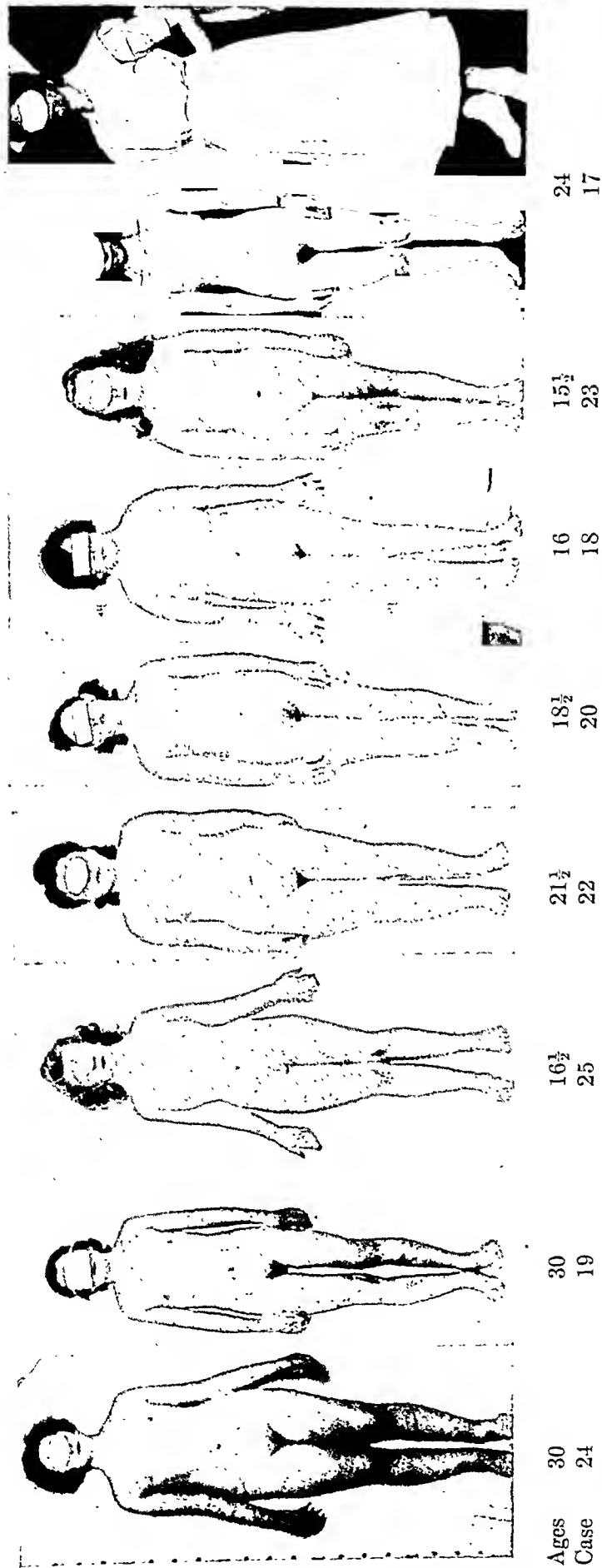


FIG. 3. Probable ovarian aplasia—no exploratory or FSH. None had menstruated

Eight patients in whom the presumptive diagnosis of congenital ovarian aplasia seemed justified by clinical characteristics. Heights in 7 ranged from 53 3/4 inches to 58 7/8 inches. One had reached 61 1/4 inches. The majority show a stocky build with the characteristic "shield" chest.

bleeding had presumably occurred from hemangiomata in the intestinal wall. At the time of the exploration he noted also that neither uterus nor ovaries were present in the pelvis. The patient subsequently died, after another severe bowel hemorrhage, the hemoglobin dropping once to as low as 15 per cent. Six of the patients had skeletal abnormalities as follows:—Madelung's deformity of the wrist in 2, osteogenesis imperfecta tarda in 1, anomalous position of a toe in 1, spina bifida occulta in 1, and abnormal development of ribs in 1.

Fifteen of the 25 cases were tested for gonadotropin output (FSH) in the urine.<sup>3</sup> All excreted abnormally high amounts. The method used was a slight modification of that published by Klinefelter, Albright, and Griswold (2). The usual range for adult menstruating females is 10–50 mouse units per day and in children of premenarchial age the range is 0–5 mouse units. Postmenopausal women excrete from 100 to 550 mouse units per day. The lowest titer in our series was 27 mouse units in a child of 13 (*case 5*, Figures 1 and 2) which was well above the normal for that age. Eleven of the remaining 14 were over 150 mouse units with the highest in *cases 2, 12* and *16*, each of whom excreted over 384 mouse units per twenty-four hours. In the present state of knowledge concerning congenital ovarian aplasia, the finding of a high urinary gonadotropin output would seem to be of paramount importance in establishing the clinical diagnosis, especially in those cases which are somewhat atypical. It should be noted that the gonadotropin excretion value drops to normal levels when the patient is under treatment with estrogens. This is illustrated by our experience in *case 9*. The level on this patient was determined while she was taking stilbestrol 42 mg. monthly and the result was 9.2 mouse units, within normal limits. She was tested again after thirty days without treatment and this time the test was positive for 192 mouse units.

Bone age studies were performed in 24 of the patients and retardation of more than two years was noted in only 4 instances. An additional 7 showed slight retardation, but in the remaining 13, the bone age corresponded to the chronological age. Thus retardation of epiphyseal closure is not the usual finding in this syndrome, which is surprising in view of the striking genital infantilism. In this connection, one should recall that in pre-adolescent eunuchoidism (in both sexes) some of the epiphyses remain ununited, even after the second decade.

The incidence of osteoporosis in our group was not as high as reported by

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<sup>3</sup> Thanks are due to Dr. Nellie Halliday, director of the Hormone Laboratory, under whose supervision these tests were performed. This laboratory is supported by grants from the Christine Breon Fund and Ciba Pharmaceutical Products, Inc., which are herewith gratefully acknowledged.



other authors. Fleischmann and Wilkins (9) noted its presence in 11 out of 30 cases, whereas our roentgenologists mentioned it in only 5 instances out of 17 with adequate radiologic study. Part of this difference in incidence may be ascribed to varying criteria of what constitutes osteoporosis in x-ray films. The age of the patient does not appear to be a determining factor.

Six of our patients were subjected to peritoneoscopy or exploratory laparotomy (Figure 1). Ovaries were either absent, or represented by vestigial



FIG. 4. Artist's drawing of appearance of pelvis at exploratory laparotomy (performed by Dr. E. W. Overstreet in *case 4*). A, Uterus, infantile in type. B, B. Fallopian tubes, tortuous and rather attenuated. C, C. Rudimentary round ligaments. D, D. "Streak" ovaries. E. Yellowish dots, probably fat in subserosa. November 1942, age 15½.

streaks (Figure 4). The youngest (*case 5*), aged 13, was explored in order to verify the diagnosis so that therapy to promote growth could be instituted well in advance of epiphyseal closure.

A word of caution should be added regarding complete acceptance of peritoneoscopic findings. In *case 2* (Figure 1), where uterus and tubes were stated to be absent, a rudimentary uterus and cervix had been palpated rectally, and the cervix was visualized per vaginum shortly after estrogen therapy was instituted. Another patient (*case 3*, Figure 1) was subjected to peritoneoscopy at 15 and 17 years of age, and no ovaries were seen on

either occasion. However, she menstruated regularly for one year after cessation of estrogen therapy, and subsequently for over three years periods have occurred irregularly at four to nine week intervals without any therapy whatever. Therefore, this patient must have possessed ovarian tissue not recognized by peritoneoscopy.

### DIFFERENTIAL DIAGNOSIS

The most important condition to be considered in differential diagnosis is primary pituitary infantilism, which likewise is characterized by shortness of stature and retarded sexual development. The outstanding differential point is the level of urinary excretion of pituitary gonadotropins which is low or absent in pituitary infantilism as contrasted with the decidedly high titer found in ovarian aplasia. There are a number of other distinctions, especially emphasized by Albright, Smith, and Fraser (1), which may be helpful in deciding whether the identical primary amenorrhea, infantile mammae, uteri, and vaginae originate from an anterior pituitary failure or a primary ovarian lack. These differences, however, are more suggestive than convincing; for instance, patients suffering from congenital ovarian aplasia are as a rule short of stature, but not as tiny as pituitary dwarfs. Furthermore, a small amount of pubic and axillary hair is usually present and more can be induced to grow by estrogen therapy, whereas the above authors contend that pituitary dwarfs do not have any sexual hair and none is produced by estrin therapy. One of the characteristic features of the syndrome discussed in this paper is a rather strong well-nourished stocky build with broad deep "shield chest," in contradistinction to the slender gracile Levi-Lorain type of skeleton typical of hypophyseal infantilism. We have previously mentioned the normal or only slightly retarded bone age which differs from the much greater retardation in pituitary dwarfism. Our findings again are in agreement with those of the authors referred to above with respect to the 17-ketosteroid excretion in the urine; this level is apt to be somewhat lower than in a normal female, but not nearly as low as in pituitary dwarfism. Thus, in 7 of our cases the 17-ketosteroid output in milligrams of the ketonic fraction per twenty-four hours was as follows: 1.3 (in a patient aged 13), 4.7, 5.1, 6.1, 6.6, 6.9, and 7.4 mg. (normally menstruating females excrete 6 to 18 mg. per twenty-four hours). Mention should be made of other evidence of congenital confusion such as coarctation of the aorta, webbed neck and cubitus valgus; these have not been reported in association with primary pituitary infantilism. Though none of the above factors can be disregarded, atypical cases occur in both categories, and in such instances especially the most helpful differential "measuring stick" is the urinary gonadotropin level. Of course, peritoneoscopy, or preferably exploratory laparotomy, or finally autopsy,

will confirm the diagnosis, small ovaries being found in pituitary infantilism in contrast to vestigial streaks or complete absence in this syndrome.

Eunuchism or eunuchoidism originating in childhood is more easily differentiated even though the primary amenorrhea and sexual infantilism are closely similar. These girls grow to be fairly tall, in contrast to the short stature characterizing ovarian aplasia. In addition, the typical disproportionately long extremities (eunuchoid proportions) and the prolonged delay in epiphyseal closure help to distinguish the two conditions. This is fortunate since FSH excretion is high in both conditions. For purposes of clinical contrast, a female castrate aged 33 years whose ovaries had to be removed at the age of 5 years is shown in Figure 1.

There should be no difficulty in differentiating the thyroid dwarfism of childhood myxedema; as a rule the shortness is more pronounced and the bone age greatly retarded. The puffiness of the features, obesity, and mental sluggishness are additional distinguishing factors; the sexual status may be retarded but on the other hand may develop precociously—menstruation occurring profusely but irregularly.

The almond-eyed slant and mental retardation of Mongolian idiots should be sufficient to exclude this condition from consideration, even though some of them are short. The typical retroussé nose, long trunk, short extremities, and well developed sexual organs rule out achondroplastic dwarfism from this discussion.

Shortness due to malnutrition, childhood diabetes, renal rickets, or congenital heart disease should not offer any diagnostic difficulties. If necessary, urinary gonadotropin determinations can be resorted to, which of course would be found normal unless ovarian aplasia were associated with one of the above disturbances. It happens that *case 14* in our series had congenital heart disease which may have contributed to her excessively short stature; and *case 10* was complicated by diabetes.

We are of the opinion that a typical case exhibiting primary amenorrhea in association with genital infantilism, short stature, stocky build with shield chest, webbed neck, and cubitus valgus constitutes a syndrome so unique that the assumption of congenital ovarian aplasia seems justified even without corroborative high gonadotropin titers or pathological verification. Unlike some congenital syndromes, notably the Laurence-Moon-Biedl syndrome, the condition is not familial (Figure 5).

It is odd that as yet no comparable condition has been recognized in males.

Finally special allusion must be made to those puzzling atypical instances of verified ovarian agenesis or aplasia where tall rather than short stature is a distracting characteristic. Wilkins and Fleischmann (9) refer to the cases of Meyer, Kuliga, and Pela, the heights being respectively 66.7

inches (169 cm.), 69 inches (175 cm.), and 70 inches (177.5 cm.); the pathological findings concerning the ovaries being "streak," "size of orange seeds," and "none seen during performance of appendectomy." One of our patients (case 6, Figure 1) was 67½ inches (171 cm.) tall and as noted peritoneoscopy revealed an empty pelvis. These 4 verified cases of ovarian aplasia, all of them tall, seem to represent a different type and may comprise a separate clinical entity. At the moment their diagnosis, unless

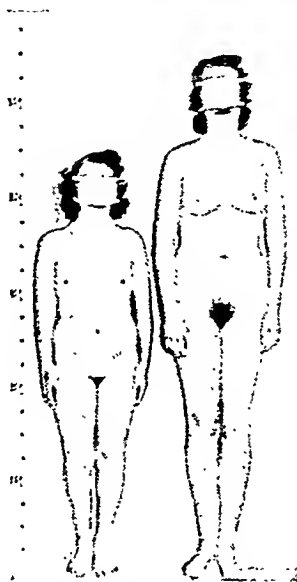


Fig. 5. Case 4, aged 15. (Verified) Standing beside normally developed sister age 17, whose height is 65 inches.

substantiated pathologically, may easily be confused with the tall eunuchoids—both types having primary amenorrhea, retarded or absent secondary sex characteristics, and high FSH excretion.

The simultaneous occurrence of congenital anomalies involving, in a definite pattern, such diverse structures as the gonads, skeletal, and cardiovascular systems, is difficult to explain on any but a genetic fault. That the precise location of the defect in a single chromosome (the exact number and relative position of the neighboring genes involved) determines the basic pattern and variations of a given syndrome (4) is an inviting concept.<sup>4</sup> It

<sup>4</sup> This concept was once elaborated by Rados in an attempt to explain the pathogenesis of arachnodactyly, a congenital syndrome involving skeletal, cardiovascular, and ophthalmic systems. It could just as well be extended to apply to other congenital syndromes such as Mongolian idiocy, hypertelorism, and the Laurence-Moon-Biedl picture.



OVARIAN AGENESIS  
M.P. 30138

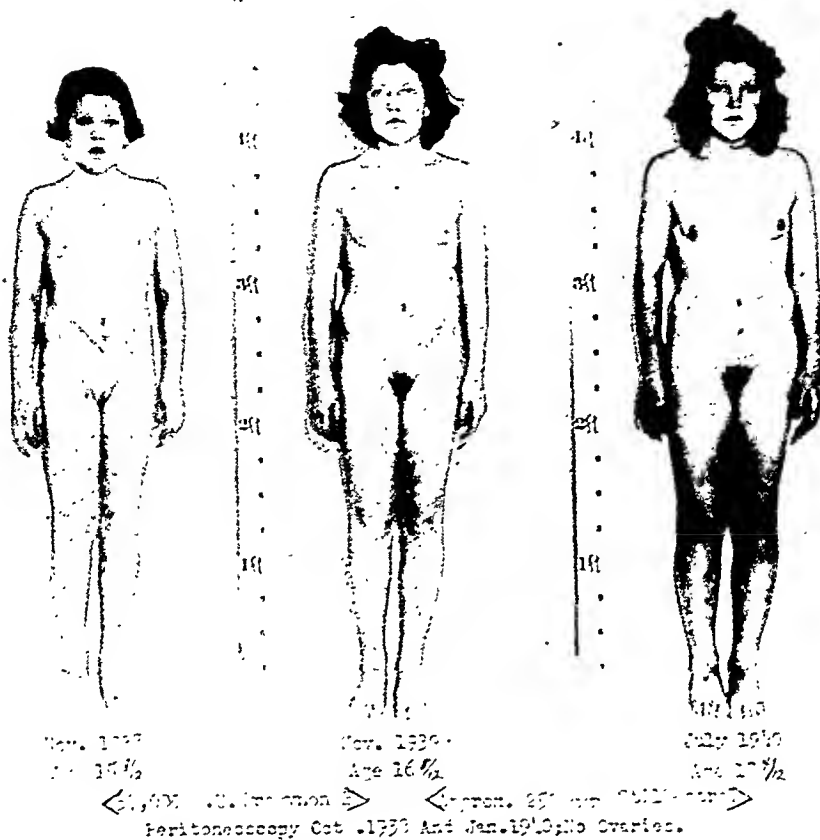


FIG. 6. Results of treatment in case 3 (verified). Note some breast development, and beginning pubic hair growth under parenteral estrogenic therapy. Note further development with marked nipple areolar pigmentation under stilbestrol given orally. In picture on left, looks fully five years younger than actual age, whereas two years later she looks at least five years older.

Estrin therapy can be counted upon to produce adequate mammary development and growth of sexual hair (Figures 6, 7, 8, and 9). The external and internal genitalia are stimulated to maturity, with the exception, of course, of the absent ovaries. These effects, together with the regular withdrawal bleedings, have a buoyant influence on the morale of the patient, who comes to regard herself as a normally acting and appearing female. This psychic side-effect should not be overlooked or underrated, indeed it is one of the most important and gratifying benefits achieved.



observed in any of our patients, even though several were under observation for many years. Indeed, unless a powerful growth stimulant is discovered, not much growth can be expected unless the proper diagnosis is made in these patients at an earlier age before the epiphyses are close to

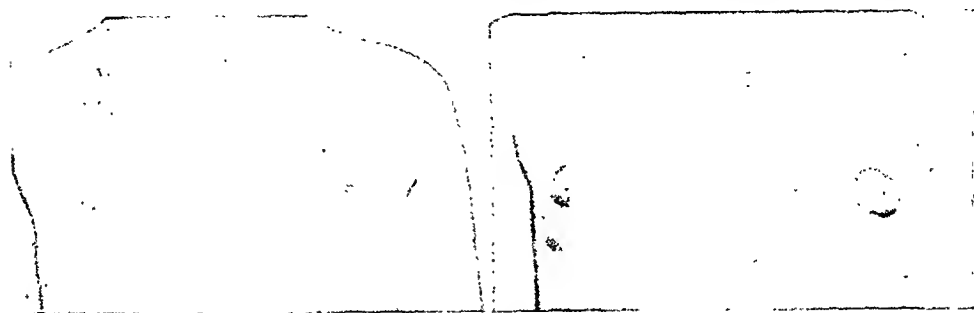


FIG. 8. Breast development in *case 24*, aged 30, resulting from one year of oral stilbestrol therapy.



FIG. 9. *Case 4* (verified), aged 15. Note growth of pubic hair from fifteen months' treatment with stilbestrol.

being united. It is to be recalled that the bone age is only slightly less than the chronological age and therefore little opportunity for growth remains if the diagnosis is delayed well into the teen-age.

#### SUMMARY AND CONCLUSIONS

Twenty-five hitherto unpublished cases of the ovarian aplasia syndrome are presented in tabular form. Six of these have been verified by explora-



tory laparotomy or peritoneoscopy. Fifteen cases were tested for urinary gonadotropin excretion (FSH) and all showed abnormally high titers. Five of these were among the 6 verified pathologically. There remained 9 of the 25 cases in which the diagnosis may be regarded as presumptive, but which we feel justified in including because of the very characteristic clinical picture. This consists of primary amenorrhea in association with genital infantilism, short stature, stocky build with shield chest and sometimes webbed neck, cubitus valgus, and other congenital abnormalities.

Severe sexual infantilism, and of course primary amenorrhea, existed in all but 2 of the 25 cases. One of these exceptions, a girl of 22 years, had well-developed breasts and had had a single spontaneous period five years before.

In only 5 of the 25 patients did the height exceed 57 inches (145 cm.) and only 1 of these was over 61 $\frac{1}{4}$  inches (156 cm.) and her height was 67 $\frac{1}{2}$  inches (171 cm.). As a rule, the stunting of growth was noticed very early in childhood.

Most of the patients displayed a sturdy stocky build with a broad shield-like chest.

Webbed neck and cubitus valgus, as emphasized by Turner, were found frequently, and about half of the 25 cases showed still other congenital abnormalities.

Hypertension was noted in only 5 instances and osteoporosis was not a noteworthy finding.

The clinical differentiation from other types of dwarfism and sexual infantilism in females is discussed. It is particularly important to distinguish this syndrome of congenital ovarian aplasia from primary pituitary infantilism.

Adequate and prolonged estrogenic therapy is specifically indicated and can be counted upon, as a rule, to produce adequate mammary development, growth of sexual hair, more mature external genitalia, and when administered orally in cyclic fashion, regular periodic withdrawal bleedings. These benefits bolster the patient's morale, which is an important aspect of treatment. However, very little growth in height results, partly because the bone age is but slightly retarded.

The recognition of 25 cases from a single clinic suggests that this syndrome is not as rare as suggested by the few cases thus far reported.

#### SUPPLEMENTARY NOTE

Unfortunately the above article of ours had been set up for printing before the publication of the excellent paper dealing with this same syndrome by E. B. del Castillo, F. A. de la Balze and J. Argonz, entitled "Syndrome of Rudimentary Ovaries with Estrogenic Insufficiency and

Increase in Gonadotropins," which appeared in the June, 1947 number of this Journal. These authors report 8 typical cases of their own, and include a table of 20 similar proven instances published between 1912 and 1941, in other words, prior to the recognition of this new syndrome clinically and hormonically by Varney and collaborators and independently by Albright and co-workers, both publishing their findings in 1942. We regret that this noteworthy contribution from Buenos Aires appeared too late for well deserved comment in our review. Any reader interested in this new syndrome should not fail to consult the above article.

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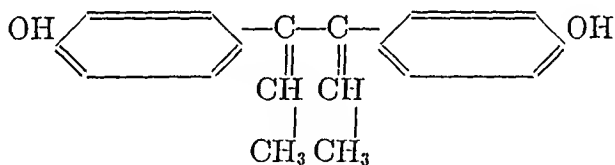
# A CLINICAL EVALUATION OF DIENESTROL, A SYNTHETIC ESTROGEN<sup>1</sup>

A. E. RAKOFF, K. E. PASCHKIS AND A. CANTAROW

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ALTHOUGH the synthetic estrogen, dienestrol, was prepared and described by Dodds and his associates (6) in 1938, it was not until 1942 that Barnes (2) carried out a clinical trial of the estrogen for the inhibition of lactation, reporting in 1944 (3) concerning its effectiveness in the treatment of the menopausal syndrome. The high oral potency of the compound, the absence of untoward or toxic effects, and improved procedures for its preparation have stimulated further clinical investigations of dienestrol.

Dodds, et al. (4, 5), in their description of the preparation of this compound, state that they had occasion to prepare pinacols of p-hydroxyacylbenzenes, which on dehydration by means of acetyl chloride and acetic anhydride gave substituted 4:4'-dehydroxydiphenylbutadienes which proved to be powerful estrogenic substances. As in the case of the stilbene derivatives, the maximum activity was found when the central chain comprised six carbon atoms giving a compound 4:4'-dihydroxy- $\gamma$ - $\delta$ -diphenyl- $\beta$ : $\delta$ -hexadiene, which was later termed dienestrol, and which can be represented by the following formula:



Dienestrol

In their assay of dienestrol on rats, Dodds et al. (4) found that on subcutaneous injection the compound was slightly less active than diethylstilbestrol which, in turn, they found to be about 2½ times more potent than estrone. They also found the diene to be quite potent on oral administration in oily solution, giving 100 per cent positive results with 3 gamma, while diethylstilbestrol gave 70 per cent positive with 1.0 gamma and 100 per cent with 5.0 gamma. In mice, Emmens (7) found dienestrol a little more potent by mouth in propylene glycol solution than by subcutaneous in-

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jection in oily solution, whereas diethylstilbestrol was only one-fourth as effective by mouth as by injection. He stated that dienestrol had the greatest oral activity, relative to subcutaneous dose, of any estrogen examined up to that time. Noble (10) found that the diene was more potent than diethylstilbestrol in inhibiting the growth of immature rats when implanted under the skin. Andersen (1) tested the potency of dienestrol, diethylstilbestrol, hexestrol and estrone in rats and mice, using single and multiple injections, and vaginal and uterine response as end points, and concluded that the marked differences in relative potency obtained by different methods made it impossible to compare the biologic effectiveness of the different estrogens by such technics.

In a clinical study of the inhibition of lactation by synthetic estrogens, Barnes (2) treated 57 patients with dienestrol, 81 with stilbestrol and 16 with hexestrol. She concluded that dienestrol gave successful results with as little as one-tenth of the dose required for stilbestrol or hexestrol. The most satisfactory results were obtained with an initial dose of 0.5 mg. twice daily, decreasing the dose by 0.1 mg. each day down to 0.1 mg. twice daily. All three synthetic estrogens were well tolerated by the puerperal women.

Barnes (3) also found dienestrol given by mouth to be highly effective in the treatment of menopausal symptoms in a group of 11 patients. With a dosage of 0.1 mg. twice daily for a period of four weeks or more, hot flashes were relieved or cured in 7 of 9 cases. Higher dosages were not tried in the two cases which failed to respond. Senile vaginitis, which was a symptom in four cases, was relieved or cured in all. None of these patients experienced nausea, vomiting or other symptoms of intolerance, and only one patient had vaginal bleeding. Vaginal smears were made once every four weeks; in 3 instances a definite estrogenic response was obtained; in 3 instances the atrophic changes in the smear were not sufficiently marked to determine a change, and in 5 instances the changes were too indefinite to warrant a conclusion.

Finkler and Becker (8) have recently reported a group of 62 menopausal patients treated with dienestrol for a period of 6 weeks to 6 months with daily doses of 0.2 to 1.5 mg. They, too, noted prompt relief of menopausal symptoms, and that untoward effects were minimal and withdrawal bleeding infrequent. No analysis was made of the minimal dosage necessary to produce a clinical response. Improvement in vaginal smears usually paralleled clinical improvement, although in a few cases the latter was achieved without any marked changes in the vaginal smear. Slight nausea was noted in only 3 cases, and in 2 of these there was some question as to whether it was due to the medication. Mild withdrawal bleeding occurred in only two instances. In a further report (9), bringing their menopausal cases to a

total of 70, no additional cases of bleeding or intolerance were noted. Sikkema and Sevringhaus (11) employed dienestrol in 21 patients and reported no nausea, emesis, nor other unpleasant side reactions with dosages up to 0.5 mg. twice daily. In 13 climacteric women who were adequately followed, satisfactory relief of symptoms was obtained with doses of 0.2 to 0.5 mg. On the basis of this series they stated that dienestrol was the most satisfactory synthetic estrogen with which they had had experience. In contrast to Finkler and Becker (8), they did not note the sense of "well-being" with dienestrol which followed some of the natural estrogens.

#### PURPOSES AND METHODS OF PRESENT STUDY

Our clinical investigations with dienestrol were undertaken with the following objectives: 1) to determine the relative usefulness of dienestrol

TABLE I. CONDITIONS TREATED—82 PATIENTS

Menopausal Syndrome	40
Postpartum Lactation	26
Secondary Amenorrhea	5
Cystic Mastitis	4
Atrophic Vaginitis	3
Ovarian Agenesis	2
Adrenogenital Syndrome	2
• Premenstrual Migraine	2
Pseudocyesis	1
Hypomenorrhea	1
Cystic Disease of Ovaries	1
Premenstrual Epistaxis	1
Essential Dysmenorrhea	1

for the various conditions in which estrogenic therapy is indicated; 2) to evaluate the estrogenic response by objective findings such as on vaginal smear response, occurrence of withdrawal bleeding and, in some instances, hormone assays; 3) to determine any possible toxic effects by repeated clinical and laboratory studies in patients taking the hormone over prolonged periods of time.

Dienestrol was administered routinely to all patients requiring estrogenic therapy as they appeared in the clinic. In each instance before treatment was undertaken the patient was thoroughly studied by physical examination, including a pelvic examination, urinalysis, blood count, vaginal smears, and whatever other laboratory procedures were indicated to establish a definite diagnosis, including in many instances assays of urinary gonadotropins, 17-ketosteroids and estrogens. In a group of twenty-five unselected patients in whom prolonged therapy was planned or anticipated, the following studies were made and repeated at intervals of one to

three months in order to determine any possible toxic effects on the blood, kidneys or liver: complete blood count, urinalysis, bromsulfalein liver function test, serum bilirubin, urine urobilinogen, cephalin flocculation and thymol turbidity test. Vaginal smears for evaluation of estrogenic effect were made at each visit. Treatment was started with a minimal dosage of hormone, such as 0.1 mg. daily by mouth, which was increased at weekly intervals until the effective dosage was reached; they were re-examined at intervals of 2 to 4 weeks. Postpartum patients who received dienestrol for inhibition of lactation were treated in the obstetric wards and, when necessary, were followed in the postnatal clinic. Ninety-six patients were started on treatment with dienestrol, 82 of whom were adequately followed; the conditions for which they were treated are indicated in Table I.

### RESULTS

**Menopausal Syndrome:** In patients with a typical menopausal syndrome dienestrol was given in slowly increasing dosage in order to determine 1) the minimal dosage necessary to obtain complete or nearly complete relief from the hot flashes and related vasomotor symptoms, 2) the minimal dosage required to change the vaginal smear from an estrogen deficiency type to one showing a slight estrogen effect, in which practically no basal layer cells are seen but the squamous epithelial cells are of the precornified type, 3) the dosage necessary to produce a good or marked estrogen effect in the smear, in which the squamous epithelial cells are chiefly of the cornified type. The results, related to the status of the patient at the onset of therapy, are indicated in Tables II, III, and IV.

TABLE II. MENOPAUSAL SYNDROME—CLINICAL RESPONSE

	Dosage Necessary for Symptomatic Relief				
	0.1 mg.	0.2 mg.	0.3 mg.	0.5 mg.	1.0 mg.
Severe Cases (13 Patients)	0	2	0	8	3
Moderate Cases (20 Patients)	4	3	0	12	1
Mild Cases (7 Patients)	3	1	1	2	0
Entire Group) (40 Patients)	7	6	1	22	4

TABLE III. OVARIAN DEFICIENCIES—SMEAR RESPONSE

	Dosage Necessary to Correct Vaginal Smear to Slight Estrogen Effect						
	0.1 mg	0.2 mg.	0.5 mg.	1.0 mg	1.5 mg.	2.0 mg.	3.0 mg.
Marked Deficiency (11 Patients)			2	7	2		
Moderate Deficiency (13 Patients)			5	4		3	1
Slight Deficiency (5 Patients)	1	1	3				
Minimal Estrogen Effect (11 Patients)	3	6	2				
Entire Group (40 Patients)	4	7	12	11	2	3	1

TABLE IV. SMEAR RESPONSE ON HIGH DOSAGE

	Dosage Necessary to Produce Good Vaginal Cornification			
	0.5 mg.	1.0 mg.	2.0 mg.	3.0-6.0 mg.
Marked Estrogen Deficiency			1	1
Moderate Estrogen Deficiency	1	1	2	1
Slight Estrogen Deficiency			1	2
Minimal Estrogen Effect	6		2	
Entire Group Tested (18 Patients)	7	1	6	4

It will be noted (Table II) that slightly more than half of the patients required 0.5 mg. of dienestrol to bring their symptoms under control but that the majority of the patients with mild symptoms and about a third of those with moderately severe symptoms could be controlled with 0.1 to 0.3 mg. daily. Only 4 patients (10 per cent) required a dosage of 1.0 mg. to control their symptoms. Improvement was generally noted within five days

but it is to be emphasized that many patients, as is the case with other orally effective estrogens, did not receive maximum benefit for 2 to 3 weeks, so that it is neither wise nor necessary to increase the dosage too rapidly.

The dosage necessary to obtain a vaginal smear response was in most instances considerably higher (Table III) than the dosage necessary to obtain a good clinical response. Seventeen (43 per cent) of the patients required 1.0 mg. or more to obtain a minimal estrogen effect, one patient requiring as much as 3 mg. The average dosage required to obtain a vaginal smear response in the entire series was 0.77 mg., while a good clinical effect was obtained with an average dosage of 0.43 mg. daily.

In 18 patients (Table IV) the dosage was gradually increased further to determine the minimum amount necessary to obtain a marked estrogenic effect on vaginal smear, as indicated by the presence of large numbers of cornified cells. In patients who started with a minimal estrogen effect, i.e., chiefly precornified cells, it usually required 0.5 mg. daily to obtain a full effect; in patients with initial estrogen deficiency smears, considerably higher dosages were required, up to 6.0 mg. daily in one case. The average dosage for this group was 1.8 mg. daily, or about four times the average amount necessary to obtain a clinical response. In individual cases this ratio was as high as 12:1.

The improvement in other symptoms associated with the menopausal syndrome, which are more difficult to evaluate, such as nervousness, irritability, headache, depression, arthralgia, etc., paralleled our experience with other estrogens given in doses which afforded relief from the vasomotor symptoms. There were 3 patients in this group with symptoms attributable to a marked atrophic vaginitis. These patients required daily 1.0, 2.0 and 3.0 mg., respectively, to obtain relief and satisfactory vaginal cornification. The dosage was considerably higher than that which they required for improvement in their systemic manifestations. Six other women with atrophic vaginitis not associated with other symptoms were treated with dienestrol locally, using a vaginal cream containing 0.1 mg. dienestrol per cc. With the daily intravaginal administration of 5 cc. (0.5 mg.) excellent therapeutic and vaginal smear response was obtained within a week.

Dienestrol was very well tolerated by all menopausal patients. None complained of nausea, vomiting or other gastro-intestinal symptoms which could be attributed to the drug, nor were there any other evidences of intolerance sometimes noted with other synthetic estrogens, such as increased headache, vertigo, etc. One patient developed a skin rash soon after starting dienestrol, which persisted, however, after withdrawal of the drug and was thought to be a neurodermatitis.

Despite the prolonged period of treatment of most of these patients,



uterine bleeding during therapy or on withdrawal was uncommon (Table V). It occurred in only 2 patients (5 per cent) who were receiving therapeutic dosages of dienestrol, and in 2 patients who were receiving increased dosages of dienestrol for the purpose of testing the vaginal smear response. It will be noted (Table V) that in three of these patients irregular bleeding had occurred prior to dienestrol therapy, either of functional origin or following administration of other estrogens. Patients of this type are not good candidates for estrogen therapy. Further bleeding always arouses the suspicion of malignancy and often requires diagnostic curettage to rule out this possibility with certainty. This procedure became necessary in two of

TABLE V. BLEEDING IN MENOPAUSAL WOMEN WITH DIENESTROL

Patient	Clinical Status	Dienestrol Therapy Preceding Bleeding	Remarks
<i>R.G.</i> Age 46	Mild menopausal syndrome	0.1 mg. daily for 6 months	Previous irregular bleeding on occasion. D & C negative
<i>M.M.</i> Age 32	Surgical castrate, supravaginal hysterectomy, severe menopausal syndrome	0.5 mg. daily for 6 weeks	Previous bleeding with premarin 1.25 mg. and stilbestrol 0.5 mg.
<i>M.F.</i> Age 48	Severe menopausal syndrome following radium for functional bleeding	0.1 to 0.5 mg. for 6 months. Gradually increased to 2.0 mg. over additional 3 months	Subsequent D & C showed scant curettings
<i>A.T.</i> Age 60	Severe menopausal syndrome for 6 years	0.1 to 0.2 mg. for 5 months, 0.5 to 1 mg. for 2 months	Slight spotting after withdrawing dienestrol

these patients (*R.G.* & *M.F.*); following further spotting three months later the latter patient had a second curettage which showed an atrophic endometrium.

**Inhibition of Lactation:** Dienestrol was administered to 26 postpartum women in whom it was desired to inhibit lactation. These women were 1 to 5 days postpartum when treatment was started. They received dienestrol in a dosage of 0.5 mg. twice daily for 3 days and then 0.5 mg. daily for approximately 1 week. The results were uniformly good. In patients treated immediately postpartum, the breasts did not fill and lactation was inhibited, while in those started later engorgement subsided promptly and the breasts dried up during the course of treatment. None of the pa-

tients exhibited any evidences of intolerance to dienestrol, nor was there a single instance of postnatal hemorrhage. When seen in the postpartum clinic subsequently, resumption of menstrual function had occurred within the usual time.

**Amenorrhea:** Dienestrol was used in the treatment of amenorrhea in nine young women, the amenorrhea being associated with a syndrome suggesting ovarian agenesis in two cases, adrenogenital syndrome in two cases, pseudocyesis in one case, and secondary amenorrhea due to psychogenic or other factors in four cases. The pertinent data in these cases are as follows:

1. *J.K.* Age 22. Syndrome suggestive of ovarian agenesis. Studies showed an estrogen deficiency smear and high urinary gonadotropins. She received dienestrol 1 mg. daily for 6 months. The breasts enlarged and there was moderate development of secondary sex characters. The vaginal smear improved from an estrogen deficiency to a good estrogen effect. A blood tinged discharge was noted at times, but no frank bleeding occurred even on withdrawal of dienestrol.

2. *H.B.* Age 25. This was a typical case of ovarian dwarfism, previously treated with other estrogens. Cyclic administration of dienestrol 1 mg. daily plus progesterone, 20 mg. by injection twice weekly at the end of the cycle, resulted in cyclic bleeding, but dienestrol alone did not produce this result. The results were comparable to those previously obtained in this patient with stilbestrol or estradiol plus progesterone, except that it was possible to obtain withdrawal bleeding at times with stilbestrol alone in a dosage of 0.25 mg. daily by mouth for a month.

3. *F.P.* Age 21. This patient had an adrenogenital syndrome characterized by hirsutism, impaired glucose tolerance, secondary amenorrhea for 3 years, sterility, and moderately increased urinary 17-ketosteroids. Cyclic bleeding was induced with 0.5 mg. dienestrol plus 20 to 60 mg. progesterone, by injection, at the end of the cycle. This was continued for 6 months. Soon after treatment was discontinued this patient became pregnant and carried successfully to term.

4. *E.A.* Age 35. An adrenogenital syndrome was present associated with secondary amenorrhea for 10 years, hirsutism, impaired glucose tolerance, and increased urinary 17-ketosteroids. Bleeding was induced with dienestrol 1.0 mg. daily plus progesterone given at the end of cycle in a dosage of 20 to 60 mg. Subsequently, dienestrol was effective alone, in a dosage of 0.5 mg. daily.

5. *C.R.* Age 32. Amenorrhea in this patient was associated with pseudocyesis and was followed by symptoms of a menopausal syndrome. Vaginal smear showed an estrogen deficiency and the urinary gonadotropin value was high. One mg. of dienestrol daily was necessary to give relief from hot flashes, but bleeding could not be induced even with a dosage of 2 mg. daily for 3 months and the patient still had an estrogen deficiency smear.

6. *M.M.* Age 35. Secondary amenorrhea was present for 7 years, with no associated symptoms. Vaginal smear showed an estrogen deficiency and the urinary gonadotropin value was high. Estradiol benzoate, 10,000 rat units, plus progesterone, 20 mg. daily, each given for 3 days by injection, produced bleeding. Dienestrol, 1 mg. daily by mouth, followed by progesterone in the above dosage for one month produced no bleeding. Stilbestrol, 1 mg. daily by mouth, followed by progesterone, resulted in vaginal bleeding. Dienestrol and progesterone were again tried with no results.

7. *S.S.* Age 34. Premature menopausal syndrome with amenorrhea and hot flashes

of 5 years' duration. Stilbestrol 0.5 mg. daily afforded good relief from symptoms; bleeding could be brought on cyclically by adding progesterone late in the cycle in a dosage of 20 to 40 mg. Equal relief from symptoms was obtained with dienestrol, 0.3 mg. daily, but it was not possible to induce bleeding by adding progesterone as above.

8. *J.C.* Age 33. Secondary amenorrhea and mild hot flashes for 2 years following resection of an ovarian cyst. Vaginal smear showed an estrogen deficiency and the urinary gonadotropin value was high. Symptoms were well controlled with dienestrol 0.1 mg. daily. No bleeding occurred when the dosage was increased to 0.5 mg. and given cyclically, but was readily induced by adding progesterone, 20 mg. at the end of cycle.

9. *Y.E.* Age 28. Secondary amenorrhea of 6 years' duration, associated with hot flashes, an estrogen deficiency smear and high urinary gonadotropin. Dienestrol, 0.5 mg. daily, given in cyclic fashion produced symptomatic relief but no withdrawal bleeding. Stilbestrol, 0.5 mg. daily, produced symptomatic relief and resulted in cyclic spotting or bleeding.

**Other Conditions:** One patient with essential dysmenorrhea received dienestrol for inhibition of ovulation. A successful result, as judged by absence of pain, was obtained the first month with a dosage of 1 mg. daily during the first 21 days of the cycle; the second month, moderate dysmenorrhea occurred with this same dosage, but successful results were obtained the third month with a dosage of 2 mg. daily. It has been our experience with other estrogens that patients with essential dysmenorrhea frequently require increasing doses of estrogen to maintain inhibition of ovulation.

Four patients with cystic mastitis had previously shown improvement with cyclic administration of other orally effective estrogens. Comparable improvement occurred with dienestrol in dosages ranging from 0.2 to 1 mg. daily.

A patient with premenstrual epistaxis was a young woman, age 25, with a normal 28 day menstrual cycle, who was treated with dienestrol in an attempt to inhibit menstruation. One mg. daily for two months failed to delay the period, but inhibition was obtained, with improvement in the epistaxis, with 2 and 3 mg. of dienestrol daily. Normal cycling followed withdrawal of the hormone and the patient became pregnant the following month. This woman kept basal temperature charts during the course of dienestrol therapy. These showed absence of the usual sharp ovulatory rise during the period of dienestrol therapy, with a sharp ovulatory rise in the month when therapy was withdrawn and pregnancy occurred.

Only moderate increase in menstrual flow was noted in a patient with hypomenorrhea treated with dienestrol, 1 mg. daily in cyclic fashion. In two patients with premenstrual migraine, the results of dienestrol therapy were inconclusive, as was the response to other estrogens. Improvement in pain and menstrual irregularity occurred in the one patient with cystic disease of the ovaries (associated with high gonadotropin excretion), treated with dienestrol, 0.5 mg. daily, in cyclic fashion.

**Toxicity Studies:** None of the patients receiving dienestrol exhibited any clinical evidence of toxic effects attributable to the drug, nor were any patients intolerant to the medication. Nausea, vomiting or headache, which are common symptoms of intolerance to some estrogens, were not encountered. As previously mentioned, there was only the one instance of skin rash and this was probably not caused by the drug.

The laboratory studies in the twenty-five patients receiving protracted dienestrol therapy also proved negative for any toxic effects. These patients received dienestrol continuously in doses of 0.1 to 3 mg. daily (average 0.82 mg.) for periods ranging from 4 to 16 months (average 7.3 months). The patients ranged in age from 18 to 65 years and included various syndromes of ovarian deficiency. The following complications were also present: thyrotoxicosis in 3 cases, essential hypertension in 4 cases, arthritis in 2 cases, functional hepatic impairment in 1 case, diabetes in 1 case, pyelitis in 1 case, duodenal ulcer in 1 case, hayfever in 1 case. No significant alterations were noted in the hemoglobin, erythrocytes, or leucocytes during the course of treatment. Urinalysis also failed to show any significant changes. In 24 patients beginning treatment with normal liver function no significant alterations were noted in bromsulfalein excretion, serum bilirubin, or urine urobilinogen. One menopausal patient with a history of previous hepatitis started treatment with 5 per cent retention of bromsulfalein at 30 minutes, a serum bilirubin of 0.4 mg. per cent and slight increase in urine urobilinogen. During the course of treatment with dienestrol (0.2 to 0.5 mg. per day) these values remained essentially the same; at the last examination after 10 months of treatment the bromsulfalein test showed dye retention of 5 per cent at 30 minutes, the serum bilirubin was 0.7 mg. per cent and the urine urobilinogen was unchanged. Cephalin-cholesterol flocculation decreased in one patient from +4 to +2, and in 3 others from +2 to 0. It increased in one case from +1 to +3 with no other abnormality, and remained at +3 in one other case. The thymol turbidity test in the latter case increased from 5.2 to 8.2 while on treatment with dienestrol, 1.0 mg. for 7 months. The bromsulfalein test in this patient remained normal, urine urobilinogen fell to normal from slightly increased values, and serum bilirubin remained at 0.2 mg. per cent.

## DISCUSSION

Although many estrogenic substances are available for clinical use, none is ideal for all purposes. The choice of an estrogen depends upon many factors, including the physiologic effects desired, the route of administration, tolerance, toxicity, untoward effects, and cost. In an evaluation of any new estrogenic substance all of these factors deserve consideration.

**Physiologic Effects:** From the studies thus far reported dienestrol ap-

pears to be of particular value for the control of the vasomotor and related symptoms of the menopausal syndrome and for inhibition of lactation, whereas it is less effective than diethylstilbestrol if a proliferative effect on the endometrium or vaginal mucosa is desired. These observations suggest that dienestrol has a relatively marked inhibitory effect upon the pituitary as compared to its influence upon the end organ. This is also suggested by the findings of Noble (10), who noted that dienestrol had a stronger inhibiting effect than diethylstilbestrol upon the growth of immature rats. In a number of preliminary experiments in castrates and menopausal women we have been able to inhibit excessive gonadotropic excretion in some but not in all instances. This point is being studied further.

**Route of Administration:** Dienestrol appears to be unique in that its oral potency is as great as, if not greater than, its potency by injection when tested in the mouse and the rat, as reported by the English workers. We have been able to confirm this observation in the mouse in our laboratory. In some preliminary observations in castrates and lactating women we have found dienestrol to be effective when given parenterally in oil or in aqueous suspension but not as effective as similar doses given by mouth. When given by injection or by mouth, dienestrol does not appear to have a prolonged action, such as has been noted with monomestrol when given by injection. In mice and rats we have found that estrus lasts for an average of 3 days following a parenteral or oral dose of ten times the minimal estrogenic dose. Preliminary studies of the metabolism of dienestrol have shown that when injected into bile-fistula dogs a considerable proportion of the estrogenic activity (more than 20 per cent) can be recovered in the bile. No observations are as yet available on the blood concentration or urinary excretion following its administration.

Dienestrol is apparently active when applied locally to the vaginal mucosa of humans, as indicated by the excellent cornification obtained in patients with atrophic vaginitis. Its relative efficacy in this respect as compared to other estrogens remains to be determined.

**Tolerance:** It has been the consensus of several clinical observers that dienestrol is one of the best tolerated of all estrogens. It may be given in full dosage with but little fear of nausea, vomiting, headache, skin rash or other symptoms. Our studies indicate that even when given over a long period of time there are no significant effects on the blood, kidneys or liver or any other evidence of toxicity.

Although bleeding on withdrawal or during the course of dienestrol therapy has been significantly less in menopausal women treated with dienestrol than with other estrogens, it has occurred in a sufficient number of instances in our own experience and in the reports of others to indicate

that this is the chief untoward effect to be guarded against. Because dienes-trol is so well tolerated and because withdrawal bleeding is reported as uncommon there is a real danger that dienestrol will be administered too freely and in too high dosage to menopausal women, with the result that withdrawal bleeding will be induced. In this study we showed that although bleeding occurred only twice in 40 patients with minimal effective therapeutic dosages it was induced in 2 more of 18 patients by further increasing the dosage. Once such bleeding begins the fear of malignancy is raised and frequently diagnostic curettage is necessary to rule it out. It is still essential to follow the rule of keeping patients on the minimal effective dosage and to withdraw therapy as soon as feasible.

Cost: Improved methods in the synthesis of dienestrol have made its commercial production less expensive than by the original process described by Dodds and his associates (4, 6). Dienestrol has therefore become available as a relatively inexpensive estrogen.

### CONCLUSIONS

Dienestrol is a potent estrogen which is highly effective when given orally. It is more potent than diethylstilbestrol for inhibiting lactation. It is of approximately the same range of potency as diethylstilbestrol for the control of the vasomotor symptoms of the menopause. It is less effective than diethylstilbestrol in inducing marked estrogenic response of the endometrium or vaginal epithelium. Withdrawal bleeding does not often occur with commonly used doses of dienestrol. Even in high dosage, dienestrol is well tolerated when given for prolonged periods of time and does not give rise to toxic effects. Dienestrol is considered to be a particularly useful estrogen for the treatment of the menopausal syndrome or for the inhibition of lactation.

### SUMMARY

The clinical response to dienestrol (4:4' dihydroxy- $\gamma$ - $\delta$ -diphenyl- $\beta$ : $\delta$ -hexadiene) was evaluated with reference to symptomatic improvement, objective response, and evidences of toxicity in 82 patients with various conditions in which estrogen therapy was indicated.

In 40 menopausal women, dienestrol afforded excellent relief in every instance, the minimal required dosage ranging from 0.1 to 1.0 mg., with an average of 0.43 mg. daily. However, the average dosage required to transform the deficiency smear to one showing slight estrogen effect was 0.77 mg., while the production of a marked estrogen effect required daily doses up to 6.0 mg. (average 1.8 mg.). Bleeding during the course of treatment or on withdrawal occurred in only two patients on ordinary therapeutic dosages and in two other patients with higher dosages.

On local application to the vagina in 6 cases of atrophic vaginitis good

results were obtained with a vaginal cream containing 0.1 mg. per cc. (0.5 mg. per dose) while in 3 similar cases doses of 1 to 3 mg. were required by mouth.

In 9 younger women with primary or secondary amenorrhea associated with primary ovarian deficiency it was difficult to induce withdrawal bleeding even with a relatively high dosage of dienestrol, which is considerably less effective than diethylstilbestrol in this respect.

In 26 postpartum patients, inhibition of lactation and breast engorgement was readily obtained with a dosage of 1.0 mg. daily for 3 days and 0.5 mg. thereafter for one week.

These observations suggest that dienestrol, given orally, has a relatively marked inhibitory effect on the pituitary and a relatively weaker estrogenic effect on the endometrium and vaginal mucosa.

No symptoms of intolerance to dienestrol were noted in any patient even in high dosage. In 25 patients treated for 4 to 16 months (average 7.3 months) no evidence of toxicity was noted nor any significant changes in blood count, urinalysis, or liver function tests.

It is concluded that dienestrol is a particularly useful estrogen for the treatment of the menopausal syndrome and for the inhibition or suppression of lactation.

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# A SIMPLE QUANTITATIVE COLORIMETRIC METHOD FOR ESTROGENIC STEROIDS

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MANY colorimetric methods for determining natural estrogens have been described, most widely used of which is the reaction described by Kober (1931) (4). Although supposedly specific for estrogenic steroids, this reaction has been shown by Mather (1940) (6) to give similar positive tests with five times as much androsterone.

Kober (1931) (4) noted that a red color was obtained upon the addition of concentrated sulfuric acid to estrone, heating, diluting with water and reheating. However, an intense greenish fluorescence was also present which interfered with the quantitative colorimetric determination by visual methods. He found that addition of phenol quenched the fluorescence and permitted a more accurate determination of the red color.

The Kober test is a two phase reaction. The first is heating with phenol-sulfonic acid, which produces a yellow colored solution, and the second phase is dilution with water or dilute sulfuric acid and reheating, which produces a red colored solution. Various modifications have been described by Cohen and Marrian (1934) (3), Cartland et al. (1935) (2), Pincus et al. (1936) (7), Venning et al. (1937) (9), Kober (1938) (5), Bachmann (1939) (1) and Szego and Samuels (1943) (8).

In the present work a re-evaluation of the reaction with concentrated sulfuric acid has been carried out and conditions set forth in which an optimum red color is obtained using only sulfuric acid. The greenish fluorescence, although present, has been found to offer no interference when photoelectric colorimeters are utilized.

## MATERIALS AND METHODS

Crystalline estrone (m.p. 254–256°C.), estriol, and  $\alpha$ -estradiol were used. All colorimetric values were obtained with a Beckman spectrophotometer. The reagents used were sulfuric acid, c.p., and absolute ethanol. All heatings were performed in a boiling water bath. No particular precautions were taken to maintain anhydrous conditions during the heating periods.

All dilutions of sulfuric acid are expressed on a volume to volume basis with the volume of sulfuric acid indicated first, e.g., (90+10) means 90 ml. of concentrated sulfuric acid was mixed with 10 ml. of distilled water.

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## EXPERIMENTAL RESULTS

## 1. Effect of Sulfuric Acid Concentration at Time of Second Heating:

A solution of 800 mg. of crystalline estrone dissolved in 2 cc. of absolute ethanol was heated with 20 cc. of sulfuric acid, c.p., for 2 minutes at 100°C. Two cc. aliquots of this solution were diluted with 10 cc. of the indicated dilutions of sulfuric acid, reheated for 3 minutes at 100°C., cooled and absorption curves obtained (Fig. 1). These curves demonstrate the diphasic

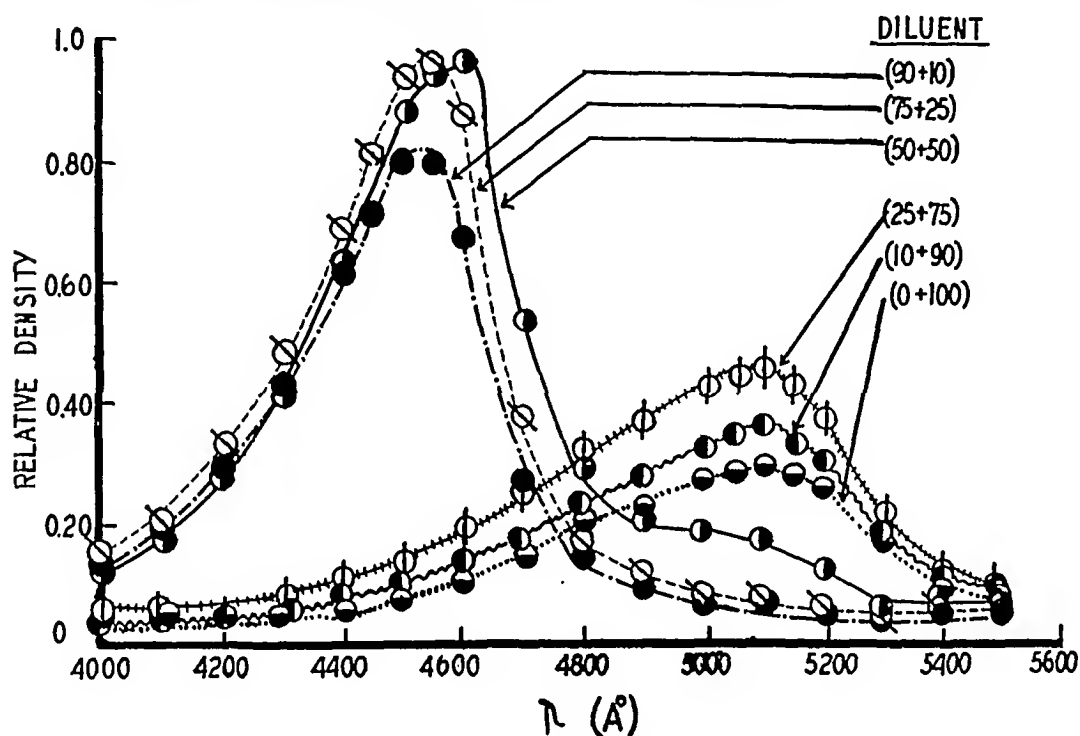
EFFECT OF  $H_2SO_4$  CONCENTRATION AT TIME OF SECOND HEATING UPON COLOR FORMATION WITH ESTRONE.

FIG. 1

nature of the reaction. With sulfuric acid (90+10) as the diluent for the second heating a maximum is obtained at 4520 Å with little or no absorption in the green region. As progressively more dilute sulfuric acid solutions are used, the absorption in the blue region decreases, and absorption in the green increases. With sulfuric acid (25+75) the maximum absorption occurs at 5100 Å while the absorption at 4520 Å has dropped to a minimum. More dilute sulfuric acid (10+90) and distilled water when used as diluent for the second heating, yield curves similar to those obtained with sulfuric acid (25+75) but the intensity of the red color is reduced. Thus maximum red color formation is obtained by using sulfuric acid (25+75) for dilution before the second heating.

## 2. Effect of Duration of First and Second Heating Upon Color Density:

Since the reaction is essentially a two phase phenomenon the conditions necessary for obtaining maximum color development were studied by varying the times of first and second heating. Samples (0.4 cc. each) of alcoholic solutions of estrone and estriol containing 40  $\mu$ g. were treated with 2 cc. of

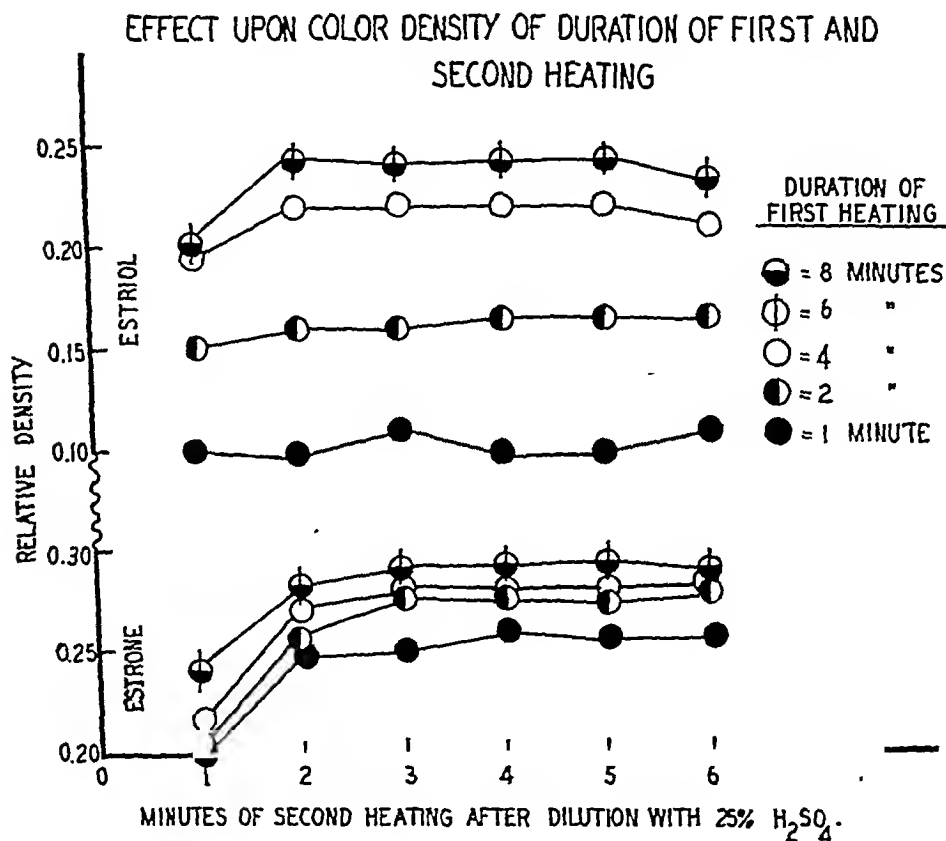


FIG. 2

sulfuric acid and heated at 100° C. for 1, 2, 4, 6, or 8 minutes. All tubes were then diluted with 8 cc. of sulfuric acid (25+75) and heated a second time for the various intervals indicated in Figure 2. Thus for each time of first heating there would be six different times of second heating. The results show that for both estrone and estriol maximum color formation is obtained with a 6 minute first heating, and a 3 minute second heating after dilution. Slightly longer times of first and second heating do not change the maximum values.

### 3. Absorption Spectra of Color Obtained with Estrone Treated by Various Modifications of the Kober Test:

40  $\mu\text{g}$ . of estrone were treated by various modifications of the Kober test, namely the Szego-Samuels (1943) (8) utilizing guaiacolsulfonic acid, and the Venning et al. (1937) (9) modification using phenolsulfonic acid and the present modification. The absorption spectra of the resulting solutions were obtained (Fig. 3). These absorption curves demonstrate that no

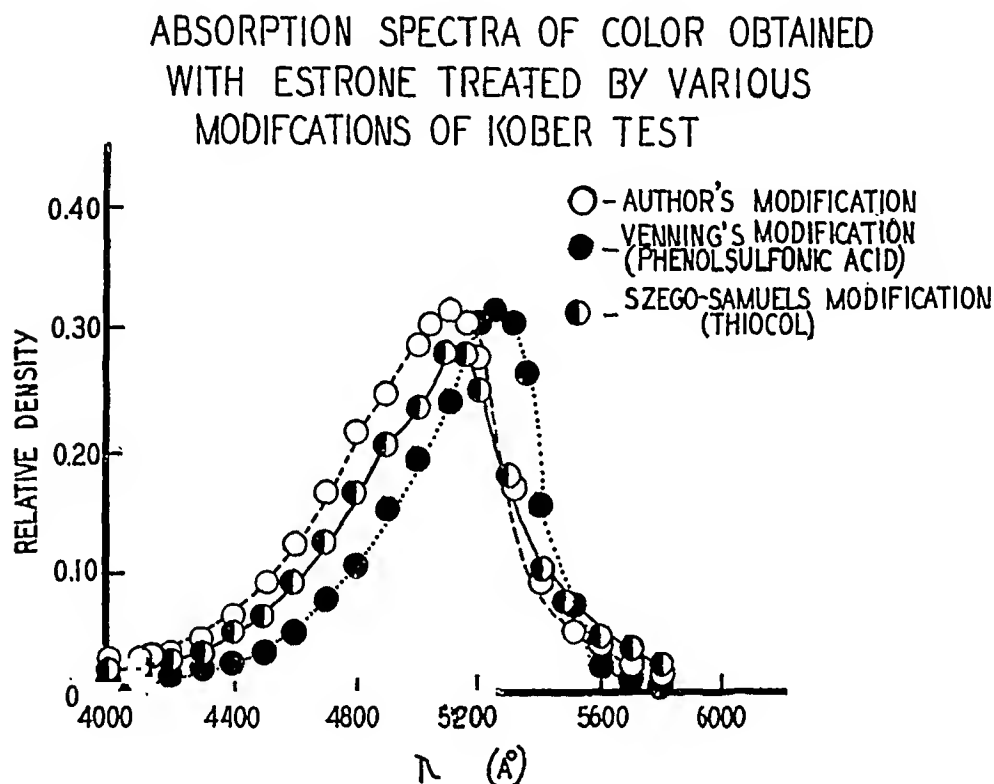


FIG. 3

particular advantage is derived from utilization of phenol reagents since the color intensity obtained with the methods using phenol reagents is of similar magnitude to that obtained with ordinary sulfuric acid. In the case of the phenolsulfonic acid reagent the peak absorption occurs at 5250  $\text{\AA}$  whereas in the other two cases the peak absorption is at 5100  $\text{\AA}$ .

There is a distinct visual difference in the solutions because phenol quenches the green fluorescence of the estrogenic steroids. Measurement of the fluorescence of the final solutions obtained by the various methods was carried out with a Klett photofluorimeter. The relative values for fluorescent intensity was 6 units for the method using guaiacolsulfonic acid; 7 units for the phenolsulfonic acid method; and 103 for the present modification. These data demonstrate the quenching effect of the phenol reagents upon the fluorescence.

#### 4. Stability of Color:

A set of tubes containing estrone treated by the present method was kept at room temperature and readings were taken at  $\frac{1}{2}$  hour intervals. Up to a period of four hours no change in color density was noted.

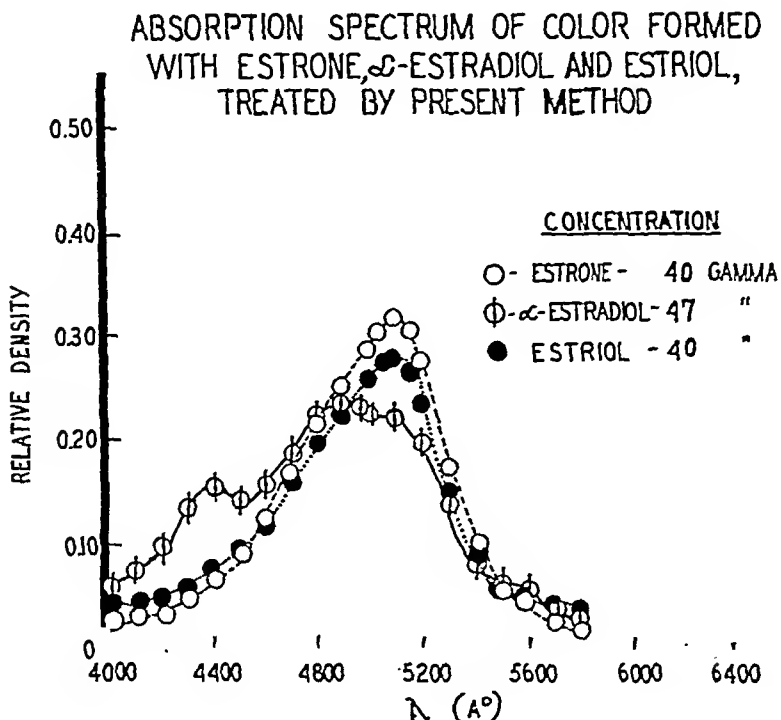


FIG. 4

#### 5. Comparison of Colors Formed With Estrone, Estriol and $\alpha$ -estradiol:

Estrone (40  $\mu$ g.), estriol (40  $\mu$ g.) and  $\alpha$ -estradiol (47  $\mu$ g.) were treated by the present modification of the Kober reaction. Absorption curves were obtained (Fig. 4). These curves show that the color formed by estrone and estriol are similar, but differ somewhat in intensity.  $\alpha$ -estradiol, however, shows a different absorption spectrum having peaks at 4400  $\text{\AA}$ , 4900  $\text{\AA}$  and 5100  $\text{\AA}$ . However, calibration curves prepared for each of these materials using the absorption at 5100  $\text{\AA}$  show adherence to Beer's law between the concentration of 5-60  $\mu$ g. (Fig. 5). The slope of each differs, being 0.725, 0.625 and 0.45 respectively for estrone, estriol and  $\alpha$ -estradiol.

#### 6. Description of Method:

Based on the foregoing observations the following procedure has been devised for the determination of natural estrogens:

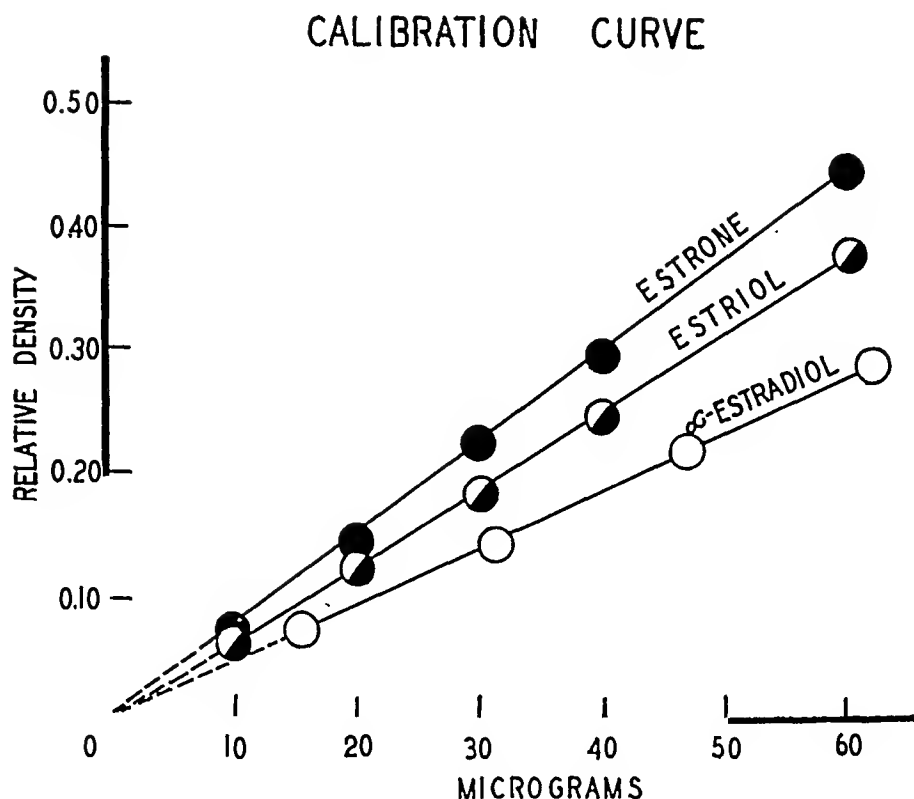


FIG. 5

- a) Place 0.4 cc. of alcoholic solution of sample containing 10–50  $\mu$ g. of estrogen in a test tube.
- b) Place tube in ice bath and add 2 cc. of concentrated sulfuric acid. Stir thoroughly keeping tube in ice bath.
- c) Place tube in boiling water bath for 6 minutes.
- d) Remove tube, add 8 cc. of (25+75) sulfuric acid and reheat in boiling water for three minutes.
- e) Cool and read color in photometer using a 5100 Å filter.

#### DISCUSSION

Kober (1931) (4) in his original paper noted the formation of a red color upon the addition of concentrated sulfuric acid to estrone, heating and diluting with water, but stated that the color formed was too weak for colorimetric measurement. He also noted that an intense greenish fluorescence was present which interfered with colorimetric readings but which was quenched by addition of phenol with an increase in the intensity of the red color. We believe that our data (Fig. 3) show that this increase in intensity is only visually apparent due to quenching of the green fluorescence and that it is not a real increase when measured photometrically.

The temperature of 100°C. for heating was arbitrarily chosen since it is easiest to control. However, lower or higher temperatures would give similar results provided the time of heating was prolonged or shortened corre-

spondingly. This was indicated by the fact that, after the first heating and dilution, similar color intensities were obtained by reheating for 3 minutes at 100° C. or allowing the solution to remain at room temperature for one hour. The advantages of this method are:

1. Reproducibility of the data with different batches of sulfuric acid.
2. Stability of final solution.
3. No special reagent.
4. Anhydrous condition and special manipulations are unnecessary.

#### SUMMARY

A modification of the Kober reaction which omits the phenol reagent is described and its experimental basis is given. Simplicity, rapidity and reproducibility of data are claimed. The method is as follows: Add 2 cc. of sulfuric acid, c.p., to the sample dissolved in 0.4 cc. of ethanol and heat the tube in a boiling water bath for 6 minutes. Remove the tube, add 8 cc. of sulfuric acid (25+75) and reheat in boiling water for 3 minutes. Cool and read in a photometer using a 5100 Å filter.

#### ACKNOWLEDGMENTS

We are indebted to Dr. D. A. McGinty of Parke Davis and Company for the estriol, to Dr. Oscar Wintersteiner of the Squibb Institute for the  $\alpha$ -estradiol and to Dr. Nettie Coy of the Squibb Chemical and Biological Laboratories for her cooperation.

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# EXPERIMENTAL USE OF TESTOSTERONE COMPOUNDS IN PREMATURE INFANTS

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**I**N A preliminary report Shelton and Varden (11) demonstrated that the oral administration of methyl testosterone to premature infants of both sexes, weighing less than 2000 grams, resulted in an apparent increase of expected survival, with concomitant minimal initial weight loss and early and sustained weight increase throughout the period of treatment.

In an effort to obtain more conclusive data than the initial study provided, testosterone compounds were administered to a much larger group of infants on the premature wards of the Los Angeles County General Hospital, over a period of several months, according to the following procedure:

All premature infants weighing less than 2000 grams were divided into three groups in rotation of entry, with no regard for sex or weight. The first group received methyl testosterone 5 mg. daily in propylene glycol, placed directly on the tongue immediately preceding an oral feeding or gavage. The second group received 4 mg. daily of testosterone propionate intragluteally; while the third group acted as the control. The testosterone was started at the end of the twelfth hour and was continued for three weeks. With the exception of the testosterone, all the infants received the same routine care which is available in any large premature station. A certain number in each group received oxygen or carbogen, if indicated, along with clysis or gavage when necessary. Routine ward orders for care applied to all three groups and the usual vicissitudes associated with premature care seemed to be present in all groups. A chart was devised by which the weight, alertness, type of stool, amount of lanugo, size of the clitoris and penis, and number of erections could be followed on each infant every day. A special registered nurse limited herself to this work, so that observations by the same individual were made from day to day throughout the experiment. For obvious reasons, it was decided that blood and urine studies were not justified in such an experiment, and that the length of time required to regain the birth weight and that required to gain to 2500 grams would be used as the ultimate basis for evaluation of treatment.

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At one interval during the period of observation the premature wards were beset with an epidemic of severe bronchiolitis. Upon review of the statistics, however, it was found that there were as many controls as those treated who had suffered from this disorder, and it was decided to include all of the infants in the final results with no regard for the obvious overall impediment of their weight gain and general development.

Treatment was begun in all infants at the end of 12 hours. Since it was felt, however, that an additional 36 hours were required before an effective level of testosterone could be expected, only those infants surviving 48 hours are included in this study.

**Results**—During the five-month observation period there were one hundred and twenty-four entries to the premature wards. Of these, twenty were stillborn, twenty-three did not survive beyond the first forty-eight hours; and seven died while on testosterone after the first forty-eight hours. Of these, two were in the control group, three in the testosterone propionate group, and two on methyl testosterone. Post-mortem examinations revealed congenital defects not compatible with life in two, intracranial hemorrhage in one, and non-specific "cardio-respiratory failure of the premature" in the remaining. Although in the original work by Shelton and Varden (11) it was felt by the observer closest to the infants and by the premature nurses that there was objective evidence of greater activity and maturity in the treated infants, no marked difference in behavior between the controls and the treated infants was observed in this series. Because of the epidemic and inadequate help, however, it is possible that the general vigor of the infants was much more difficult to evaluate in this series than in the first group, which were observed by a number of individuals in a private institution.

No untoward effects were observed in either of the two treated groups. It had been speculated by the authors that hoarseness in the cry of the treated females might be one of the signs of an untoward effect. As much cry as is expressed by a premature did not reveal any alteration in pitch in either sex in the treated group. There was no abnormal hair growth and no objective enlargement of the genitalia in either sex.

The seventy-four cases were divided into three groups: twenty in the control group, thirty in the methyl testosterone group, and twenty-four in the testosterone propionate group. These infants were further divided by birth weight into two classifications, 1000 to 1500 grams and those weighing between 1500 and 2000 grams. As 2500 grams is the American Academy of Pediatrics accepted level for normal infants, it was decided to tabulate the days required to regain birth weight, and the days required to regain to a 2500 gram level. The statistics reveal a significant difference between the controls and testosterone-treated infants.



Table 1 shows that the control infants in the 1000 to 1500 gram weight groups required on an average of 14.7 days to regain their birth weight, whereas those on methyl testosterone in the same weight group required only an average of 9.0 days, and those on testosterone propionate 7.5 days. Thus, there was a 50 per cent reduction in the treated over the control group in time required to regain birth weight. In analyzing the 1500 to 2000 gram group, it is shown that the controls required 11.9 days to regain their birth weight, in contrast to 7.8 days for the methyl and 9.8 days for the

TABLE 1

Weight	Treatment	Number of Cases	Days to Regain Birth Weight	Days to Gain to 2500 Gm.
1000 to 1500 Gm.	Control	6	14.7 days	58.8 days
	Methyl Test	6	9.0 days	56.9 days
	Test Prop.	4	7.5 days	55.0 days
1500 to 2000 Gm.	Control	14	11.9 days	42.1 days
	Methyl Test	24	7.8 days	32.6 days
	Test Prop.	20	9.8 days	35.1 days

testosterone propionate infants, which is also an apparently significant trend. In considering total hospital stay, those treated with testosterone compounds again demonstrated a variance from the control groups. The 1000 to 1500 gram control group required 58.8 days to gain to 2500 grams in contrast to 56.9 days for the methyl testosterone group and 55.0 days for those infants on testosterone propionate. The 1500 to 2000 gram controls took 42.1 days to reach 2500 grams, in contrast to 32.6 days for the methyl testosterone infants and 35.1 days for those on testosterone propionate.

An even more significant indication of the benefits obtained from the use of testosterone compounds is shown in Table 2. Here four sets of twins were employed. In three sets the larger of the two (the one with the greater chance for survival) was used as the control, while the smaller was given testosterone. All four of the twins on the testosterone compounds, who, theoretically should have taken longer to regain their birth weight, did so in a much shorter period than their larger, more mature siblings, who were used as controls. In three of the cases the birth weight was maintained from the start, while in the fourth there was an approximate 50 per cent reduction in the anticipated number of days required to regain the birth weight. Again the days required to gain to 2500 grams shows a reduction in the

win treated with testosterone in three out of the four, while in the fourth not only two days longer were required to bring the smaller infant to maturity.

It is quite likely that the early weight gain or at least the maintenance of birth weight, so commonly observed in these infants, is due to salt and water retention. However no instance of edema was observed. The weight gain up to 2500 Gm. apparently represents a true somatic increment since,

TABLE 2

Twins	Birth Weight	Type Treat.	Days to Regain Birth Weight	Days to Gain to 2500 Gm.
S. #1	1956 Gm.	Control	17 days	32 days
S. #2	1871 Gm.	Test. Prop.	No days—maintained B. wt.	22 days
M. #1	2041 Gm.	Control	9 days	25 days
M. #2	1816 Gm.	Test. Prop.	5 days	17 days
B. #1	1531 Gm.	Control	7 days	50 days
B. #2	1276 Gm.	Methyl. Test.	No days—maintained B. wt.	52 days
P. #1	1446 Gm.	Control	13 days	53 days
P. #2	1531 Gm.	Test. Prop.	No days—maintained B. wt.	41 days

in those infants followed after withdrawal of the hormone no sharp weight loss was seen.

The dose of both methyl testosterone and of the propionate were chosen arbitrarily, and are probably higher than those required to obtain a maximum metabolic effect. In comparing the methyl testosterone with the testosterone propionate, no significant differences could be observed. Testosterone propionate perhaps did offer advantages over oral administration in the accuracy of dosage, but the ease of administration of methyl testosterone in propylene glycol, either on the tongue or in the feeding, and the freedom from the infection hazard is worthy of consideration.

#### DISCUSSION

It is now generally accepted that testosterone propionate and methyl testosterone cause a decline in urinary nitrogen, reflected in the urea frac-

tion, unaccompanied by an increase in the plasma protein, non-protein nitrogen, urea, or hemoglobin concentrations. As there is no change in the amount of fecal nitrogen, it is evident that this retention of nitrogen must have some tissue building significance. There is also a decline in urinary sodium, associated with a somewhat smaller decline in urinary chloride; a decline in urinary potassium and total urinary volume. The evident end result of this is in water retention and a general protein anabolism, noticeable in the genital tissue, but more objectivized in the soma, with resultant increase in growth and muscle hypertrophy. These effects have been demonstrated by Rubinstein and Solomon in the rat (9); Kochakian and Marlin in the dog (5), (6), (7); Kenyon, et al. in the adult (2), (3), (4); and by Wilkins, Fleischman, and others, in immature children (12). Shay, et al. (10) found that testosterone stimulated growth in normal female rats, but no effect on growth was observed by them when male rats were injected. From this it might be inferred that the male, when surfeited with autogenous hormones, is incapable of further response, but the prematures of both sexes, who are devoid of this physiological activity, might profit by such stimulation at the most crucial period of their existence.

It is believed that with the doses used and especially the sharp period of time during which it was administered, the action of the testosterone should have no effect on osseous development or on epiphyseal closure. McCullagh (8) has shown that small doses of testosterone propionate (15-60 mg. per week) for fifteen months caused no distinct advancement in epiphyseal age, but that doses of 105 mg. or more per week, administered over a period of eight months, did lead to an increase of about two years in epiphyseal development. Gordon and Fields (1) using doses of 20-50 mg. of testosterone propionate per week on pre-puberal and adolescent boys found no instance of premature closure of the epiphysis or of stunting of growth after extended periods of treatment. However ridiculous, some physicians continue to be obsessed with the idea that the administration of testosterone to the infants, for even a few weeks, might interfere with their future growth. For this reason, we have compared roentgenograms of the wrist and knee of the treated individuals, at intervals throughout the first year, with the controls, and, as anticipated, found no difference.

#### SUMMARY

1) Seventy-four premature infants of both sexes under 2000 grams entering the Los Angeles County General Hospital were divided into three groups: one a control; one placed on methyl testosterone, 5 mg. daily; and one placed on testosterone propionate, 4 mg. daily.

2) A distinct shortening in the time required to regain birth weight and in time required to gain to 2500 grams was noted in both groups receiving testosterone compounds.

3) Four sets of twins were included in the study, and in each instance the one on testosterone showed increased somatic development over its control sibling.

4) It is not our intention to advocate the routine use of testosterone compounds in premature infants, but to suggest such a procedure as an adjunct in the care of a selected group needing metabolic stimulation.

5) No contraindications to the use of testosterone compounds were found.

#### ACKNOWLEDGMENT

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# GOITER ON AN IODINE-FREE DIET GROWN BY HYDROPONICS, AND EXCLUDING ANY GOITER NOXA

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VARIOUS workers have experienced difficulty in finding foodstuffs sufficiently low in iodine to produce goiter. This fact has helped to revive the germ ("virus") theory of goiter (1). It seemed of interest to produce goiter in a goiter-free region with precautions against introducing any hypothetical virus. In order to obtain food free from iodine and virus it was grown by hydroponics in a disinfected greenhouse.

According to Frazier (2) goiter is not endemic in this region. Furthermore we did not allow any person or animal with goiter inside the greenhouse. The floor was freshly coated with asphalt and the whole interior, including tanks and window panes, sprayed with 5 per cent DDT and 1 per cent cresol in kerosene. The greenhouse is 25 miles from the nearest city and the water used was rain caught on a glass roof and filtered through amberlite IR4 freshly activated with aluminum ions which would remove any "goiter germ." The reagent chemicals were dissolved in water at pH 10 and filtered through freshly precipitated tri-calcium phosphate which would kill or remove any germs.

Seeds of corn, soybeans and sunflowers were sprouted in broken quartz that had been heated to 150°C in sulfuric acid (a process that would kill any germs). The seedlings were transplanted to holes in the aluminum covers of the tanks. The entering air passed through copper screen with holes 0.6 mm. in diameter, the outgoing air through new galvanized mosquito screen 10 feet from ground and the air to the roots was filtered through 2 inches of packed asbestos followed by carbon filter tubes. From these crops and pure sterile chemicals a diet of 40 per cent sunflower seed, 2.8 per cent soybeans, 40 per cent sucrose, 0.8 per cent NaCl and 16.4 per cent corn oil was prepared. This low percentage of soy beans is not goitrogenic. Six litter mate rats of a "Wistar" colony that had been isolated for 6 years from goitrous animals or humans were put at weaning on this diet. The cages were isolated in a cellophane covered 6×10×10 foot space. Three of the rats were given water re-distilled from alkali and the other three water containing 10 parts per million of iodine. At the end of 73 days the rats were anesthetized, the thyroids dissected out, placed on small round, pre-

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viously weighed cover glasses and quickly weighed on a chainomatic balance. We had previously found this method as accurate as weighing bottles for two reasons. First, a weighing bottle has a large surface to adsorb moisture; second, during the time it takes for the air in a weighing bottle to come to the temperature of the balance case some of it has been pushed out around the stopper by the water vapor from the fresh tissue.

The rats on the diet and re-distilled water had goiters weighing 41, 42 and 39 mg. per 100 Gm. body weight whereas the rats on the diet and water containing 10 ppm. iodine all had normal thyroids weighing 10 mg. per 100 Gm. body weight. It is thus demonstrated that an iodine-free diet produces goiter.

Greenwald did not present any evidence that goiter is due to a virus and in a second paper (3) borrowed Dieterle and Eugster's term "goiter-noxa" and formulated a number of criteria for iodine workers, one being that the iodine added to prevent goiter not be greater than in ordinary foodstuffs. We have analyzed food pellets used for controls by other workers and found them to contain more iodine than the water we gave our controls.

The goiters produced on the hydroponics diet in a non-goitrous region were twice as large as goiters produced in a goitrous region in rats of goitrous mothers (4).

Conclusion: Goiter was produced by a lack of iodine.

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# PHEOCHROMOCYTOMA WITH DIABETES\*

## A CASE REPORT AND DISCUSSION

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**H**YPERTENSION and glycosuria are conditions which in the vast majority of cases are not accessible to causal therapy. Yet there is a small—but important—group of patients with high blood pressure, paroxysmal or persistent, and with glycosuria, transitory or even permanent, who can find curative treatment, if the correct diagnosis of their disease is made in time. These are the patients with pheochromocytoma. The pheochromocytoma is an endocrine tumor of adrenal medullary tissue and produces epinephrine. Its active cells are chromaffin and give a brownish stain when exposed to a solution of potassium bichromate; it is from this reaction, that the name pheochromocytoma (brown cell tumor) was derived.

The adrenal medulla originates from the sympathetic nerve system and the pheochromocyte is one of the final developmental forms of the sympathogonia, the stem cell of the sympathetic nerve system. It is therefore easily understandable that only those tumors of the adrenal medulla are hormone-producing in which the differentiation of the stem cells has progressed to the stage of the pheochromocyte. On the other hand, pheochromocytoma may develop outside of the adrenal medulla wherever sympathetic nerve cells are present, as for instance in the sympathetic glands along the aorta and the carotid body. Table 1 shows the pheochromocytoma in its relation to the other non-endocrine tumors of the sympathetic nerve system.

Like most endocrine tumors the pheochromocytoma manifests itself more by symptoms of hormonal overproduction than by invasive growth and metastases, which in fact are rather rare. Its "malignancy" is due mainly to the serious effects of the overflow of its incretoric product upon the general metabolism and particularly the cardio-vascular system. But unlike the cortical tumors of the adrenals or the pancreatic islet cell tumors the symptoms of the pheochromocytoma are rather unspecific and may occur in numerous other conditions of functional or organic origin, from

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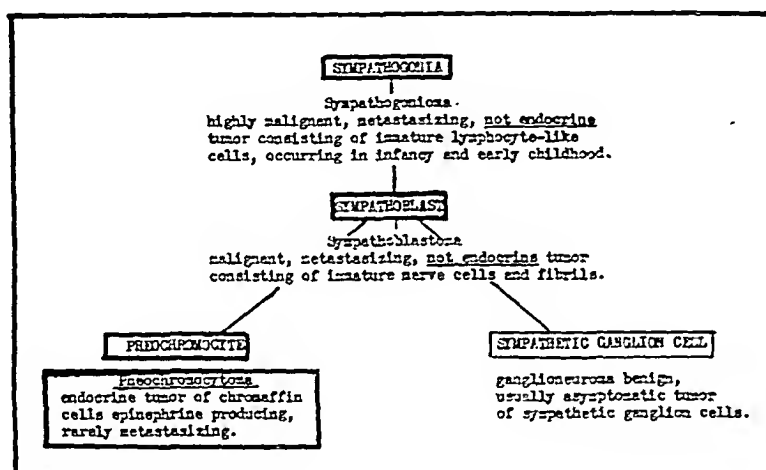
\* Clinic presented at the Annual Meeting of the American College of Physicians at Chicago, April 29, 1947.

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neurocirculatory asthenia to malignant hypertension. Therefore its clinical diagnosis is rather difficult and is made more frequently where the physician is aware of its occurrence and considers the possibility of its presence in a given case, than when a correct diagnosis is expected to be the result of a series of objective laboratory tests. The pheochromocytoma is a rare disease, but the number of clinically diagnosed and surgically cured cases has been increasing rapidly since the first description of the syndrome, and this increase is certainly not due to an increased incidence of the disease, but only to the better diagnosis.

It was more than 25 years after the discovery of the medullary hormone of the adrenals (1895) that the clinical syndrome of the medullary tumor

TABLE 1



was described by Labbé, Tinel and Doumer in 1922 (9). Humphreys (8) was able to collect 103 cases from the literature up till 1939, but most of these were postmortem observations. In 1937 Howard and Barker (7) reported 18 clinically diagnosed cases, in 1941 Biskind and co-workers (1) analyzed 33 clinically diagnosed and operated cases from the world literature, out of which 26 had been cured surgically, and in 1944 Cahill (4) reported 9 cases of his own observation. In Billings Hospital 5 cases were seen in recent years, 3 of them reported by Brunschwig, Humphreys and co-workers (2). During the 2 years 1945-1946 at least 24 individual reports of successful removal of pheochromocytoma have appeared in the world literature.

It is gratifying to note the increasing frequency with which diagnosis is made or suggested by the practicing physician out in the field instead of being left to the rear echelon of the well equipped diagnostic hospital. The



credit for the correct differential diagnosis of the case, here to be discussed, goes to the family physician who referred the patient to the hospital for further observation and treatment.

This patient's case is of special interest because among its presenting signs and symptoms were not only glycosuria but a typical diabetes. As far as we have been able to ascertain, this seems to be the second case of its kind to be reported, the first case being described by Duncan, Semans and Howard in 1944 (6).

The patient, *M.L.E.* (#228050), was a 20 yr. old white female who was admitted to the Albert Merritt Billings Hospital of the University of Chicago on 7-11-44. She had been in excellent health until about 1942 when she first noticed periodically recurrent headaches, increasing nervousness, occasional attacks of mild dizziness and hyperhidrosis, nocturia and polyuria. It was not until two years later that she was seen by a physician whom she consulted not because of her symptoms but in order to obtain a health certificate. At that time glycosuria and hyperglycemia were found and the diagnosis of diabetes mellitus was made. Her diabetes proved to be hard to control, though she did not develop acidosis or coma. Even 85 units of insulin daily were insufficient to maintain normo-glycemia or to keep the urine free of sugar. Her blood pressure in the spring of 1944 was found to fluctuate between 110/70 and 180/120.

In May, 1944, patient was under observation of the Lilly Laboratory for Clinical Research, City Hospital, Indianapolis. There the fluctuating blood pressure was confirmed. Variations from 110/50 to 170/110 were noted in two daily readings over a period of 3 weeks. The retinal vessels showed a grade 1 constriction and questionable grade 1 sclerosis. Many small retinal hemorrhages were noted in either eye and an occasional white exudate was seen. Plethysmographic examinations of fingers and toes indicated no arteriosclerosis. Electrocardiogram and teleröntgenogram of the chest were normal. Kidney function tests gave normal results. The diabetes showed again an unusually high insulin requirement and unusually wide fluctuations of the fasting blood sugar. On a diet of 200 C, 86 P, 100 F, the urine could be kept nearly sugar free but the fasting blood sugar ranged from 72 to 367 mg. per 100 cc.

On admission to Billings Hospital, the patient appeared apprehensive and complained about nervousness, headaches and excessive perspiration. Family history and history of previous illnesses were not contributory. Menstrual history was normal. The physical examination revealed a well developed female with a slight excess of dark hair on face, breast, abdomen and thighs. The patient was not certain whether or not her hair growth had increased recently. Her weight was 48.7 kg. (107 lb.), height 155 cm. (5 ft. 1 in.). The skin was moist and there was mild tremor manum and moderate hyperreflexia. The eye reflexes were normal. No exophthalmos or lid lag was noted. The thyroid was not enlarged. The chest was symmetrical and normal to auscultation and percussion. The heart appeared to be of normal size, rate and rhythm. There were no murmurs. The blood pressure was 170/120; the abdomen was soft, liver and spleen did not appear enlarged, no masses were palpated in the abdomen. The pelvic examination was normal. Fundoscopic examination (Dr. Spiro) revealed many hemorrhages of various sizes over both retinae, surrounded by irregular white exudates. The retinae appeared edematous, the veins were markedly engorged and tortuous, the arteries only slightly constricted. The crossing changes were marked.

The blood count was normal, the serology negative, the urinalysis negative for albumin but positive for sugar, the specific gravity 1.027, the sediment normal. X-ray

examinations of chest and skull were normal, intravenous and retrograde pyelograms showed no abnormalities in kidney shape or function; a small bony defect, in the right ilium near the sacro-iliac joint was observed. (This unidentified lesion was reexamined at intervals during the subsequent two years and has not changed in size.) The electrocardiogram was again normal.

Blood pressure readings were taken 4 times daily and again marked daily fluctuations were noted. Fig. 1 demonstrates the course of the blood pressure. Sodium amytal and cold pressure tests gave normal responses. The kidney function was studied extensively with urea-, inulin- and diodrast-clearances, and was found to be normal.

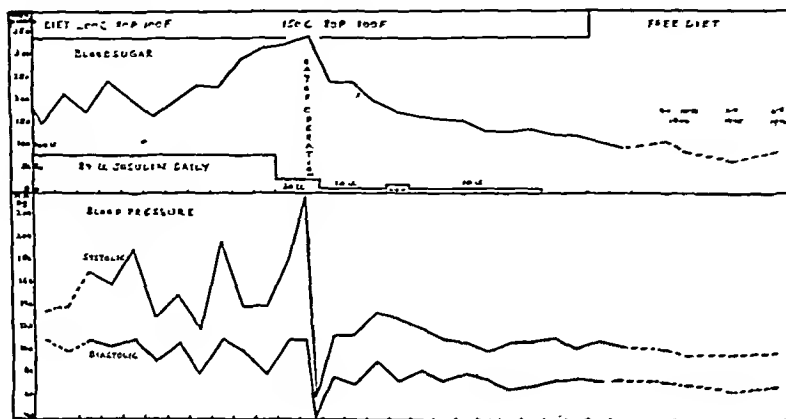


FIG. 1

The diabetes remained unstable, with fluctuations of the fasting blood sugar between 81 and 290 on a stable diet (200-80-100) and 85 units of insulin. The course of the blood sugar and the insulin dosage are demonstrated on Fig. 1. An insulin tolerance test showed an unusual response with a slight drop of the blood sugar during the first 15 minutes (from 225 to 190) and with a rapid return to a value higher than the fasting level (275) within 2 hours. Such a curve may be obtained if adrenaline is given together with or shortly after insulin. Because of the rather mild hirsutism the question of an adrenal cortical tumor was discussed. The 17-ketosteroid excretion was determined and found to be normal. The blood electrolytes were Cl 96 mEq./L, Na 136.8 mEq./L and K 4.5 mEq./L.

It was felt that pheochromocytoma was the most likely possibility in the differential diagnosis and surgical exploration was recommended. This was performed by Dr. William E. Adams on 7-24. The abdominal approach was used. Exploration of the region of the right adrenal gland revealed a mass approximately  $5 \times 3$  cm. in size which was medial and superior to the kidney and in the medial aspect of the adrenal gland. The left adrenal appeared normal. The tumor mass was freed from the surrounding tissue, the blood vessels of the tumor were clamped, and the tumor was then removed. During this procedure the blood pressure rose to 240/120 and then fell to a shock level of 60/40; under ephedrine the blood pressure returned to normal levels within a short period of time.

Morphological-pathologic examination of the tumor by Dr. E. Humphreys revealed the following: The tumor,  $3 \times 5$  cm. in diameter, consisted of rather homogenous tissue

and had a fibrous capsule. Two pieces of the tumor were immersed in 3 per cent bi-chromate solution and developed the brownish color typical of pheochromocytoma. Microscopically the tumor was composed of cells arranged in nests and cords separated by small blood vessels. In many regions, the appearance was identical with that of the normal adrenal medulla.

Post-operative course: The patient had an uneventful recovery from her operation. The blood pressure remained within normal limits throughout and was 120/90 on 5-6-46, about two years after the operation (Fig. 1). Headaches, dizziness, nervousness and hyperhidrosis have disappeared completely and permanently.

Reexamination of the fundi showed gradual disappearance of the bilateral retinopa-

TABLE 2

THE POST OPERATIVE IMPROVEMENT OF THE ORAL GLUCOSE  
TOLERANCE IN A CASE OF PHEOCHROMOCYTOMA WITH DIABETES

Date	1944 8-9		1945 2-7		1946 6-5	
Time	Blood sugar	Glycosuria	Blood sugar	Glycosuria	Blood sugar	Glycosuria
Fasting	144	0	71	0	91	0
30'	258	0	169	0	192	0
120'	282	++	209	+	172	0
180'	242	++	190	+	124	0

thy. By May 1946 the vessels of the fundi had returned to normal appearance; neither hemorrhages, nor exudates or retinal edema were visualized.

The diabetes showed the most spectacular change. Immediately after the operation the fasting blood sugar level began to drop, in spite of the fact that the daily insulin dose had been decreased. Within 10 days the blood sugar had returned to normal and the urine remained sugar free. Only 10 units insulin daily were given during that period. The patient then was permitted a free diet and insulin was discontinued. Her fasting blood sugar remained normal and her urine sugar-free. To check this apparent cure of her diabetes an oral glucose tolerance test was performed the day prior to her discharge from the hospital. This test showed a typical diabetic curve and thus revealed that though the diabetes had improved markedly, it had not been cured (Table 2). After discharge from the hospital the fasting blood sugar was found to be normal at various examinations and the urine to be sugar-free at all times. Annual glucose tolerance tests however showed the persistence of a mild diabetic state.

### COMMENT

Almost all of the typical features of a pheochromocytoma are illustrated by this patient's history: The fluctuant hypertension, the palpitations, dizziness and headaches are classical symptoms of epinephrine overactivity. Hyperhidrosis, nervousness and tremor manum are encountered frequently as associated symptoms. These, if very marked and accompanied by an elevated basal metabolic rate, may occasionally mislead to the differential diagnosis of hyperthyroidism. It should be remembered

that epinephrine itself may cause hypermetabolism. The age of the patient and the location of the tumor illustrate the fact that pheochromocytomata occur most commonly in young or middle aged persons and are more frequently found in the right than in the left side of the abdomen. The occurrence of the relatively minor fluctuations of the blood pressure during the operation reminds of the sometimes very serious complications of surgery. Manipulation of the tumor may express large amounts of adrenaline into the circulation which may suddenly precipitate an extremely high rise of blood pressure, peripheral vasoconstriction, lung edema and death. Ligation of all blood supply prior to removal of the tumor, as was done in this case, prevents this hazard. On the other hand, circulatory shock threatens after extirpation of the tumor and substitution therapy with epinephrine and cortical extract is frequently necessary for a short period postoperatively. Because of the occasional multiplicity of pheochromocytomata and the importance of examining both adrenals prior to extirpation of the diseased one, the abdominal approach, as used here, has been accepted generally as superior to the retroperitoneal dorsal method, which permits only unilateral inspection. The return of the blood pressure to a normal level and the practically complete disappearance of the retinal changes are the gratifying and common results of surgery if done in time.

It is noteworthy that, except for the diabetes, the symptoms in our patient were rather mild. None of them alone would have been indicative of a pheochromocytoma, yet the combination of all was very suggestive of the diagnosis which proved to be correct at surgery. This supports Howard's dictum that "when diabetes mellitus, hypertension and hypermetabolism are encountered, the possibility of pheochromocytoma should be considered," to which we may add that this consideration is warranted especially if the patient is of middle age or younger and if no evidence of arteriosclerosis or primary kidney disease is found. Occasionally palpation, more frequently a pyelogram or radiography, in combination with perirenal air insufflation will establish the diagnosis, but not infrequently the tumor is so small that nothing short of exploratory laparotomy will prove the presence or absence of a pheochromocytoma. This, of course, should be done only if the symptoms are suggestive, but then it is indicated, indeed. In many instances exploratory laparotomy may mean the difference between a chance for complete cure on one hand and the poor prognosis of progressive hypertension and arteriosclerosis on the other hand, with the added danger of sudden death in a hypertensive crisis.

The course and the significance of the diabetes in our patient deserves a special discussion. There are a few case reports in the literature where a typical diabetic syndrome, persistent hyperglycemia and glycosuria, was associated with pheochromocytoma, but with the exception of the case of

Duncan, Semans and Howard (6), either the diagnosis of pheochromocytoma was made only postmortem or the patient died soon after operation so that no observations about the later course of the diabetes could be made nor could any conclusion be drawn as to the relationship of the diseases. In the only case similar to ours, an insulin resistant diabetes of more than three years' duration improved to such a degree that it could be considered cured for all practical purposes. The patient did not require insulin any longer and was sugar free and normoglycemic on a daily intake of 300 Gm. carbohydrate. Here too, however, decreased glucose tolerance tests were obtained months after removal of the tumor, but the authors were inclined to minimize the significance of this "slight residual defect in the mechanism of carbohydrate disposal." In this as well as in our case the marked, rapid and persistent post-operative improvement of the disturbed carbohydrate metabolism argued strongly against the possibility that the diabetes was a coincidental and independent disease. The relatively high insulin requirement in both instances points in the direction that extra pancreatic factors were at least contributory to the occurrence of hyperglycemia and glycosuria. Overactivity of epinephrine, causing increased glycogenolysis in the liver, was most likely the initial step in this mechanism. As long as the hormone secreting tumor was present the hyperglycemia persisted. After the operation a return to normal, a *restitutio ad integrum*, seemed to have occurred, but a persistent though latent disturbance of the carbohydrate metabolism became evident under the stress of the glucose tolerance test. It seems to us that this remaining decreased carbohydrate tolerance deserves the same emphasis as is given to the marked clinical improvement. It has long been argued that prolonged hyperglycemia of non-pancreatic origin may lead to irreversible pancreatic islet cell damage and diabetes. The present stand on this problem and the evidence in favor of this hypothesis has been summarized ably by Ricketts (10), and only recently Dohan and Lukens (5) have demonstrated islet cell degeneration in cats, the blood sugar of which had been elevated for prolonged periods of time by glucose injection. The assumption, therefore, seems permissible that the prolonged hyperglycemia which was produced by the pheochromocytoma had exerted irreversible damage upon the blood sugar regulatory mechanism so that a decreased carbohydrate tolerance persisted after removal of the tumor. Unfortunately no biopsies from the pancreas were obtained in our patient. Further clinical observations will have to prove whether such damage is manifested by histological changes in the pancreas.

#### SUMMARY

A new case of pheochromocytoma with diabetes is reported and the symptomatology of this syndrome is discussed. The persistence of a de-

creased carbohydrate tolerance after successful removal of the tumor seems to give support to the theory that hyperglycemia of extrapancreatic origin may cause irreversible pancreatic islet cell damage and diabetes.

### ACKNOWLEDGMENT

The author wishes to acknowledge his indebtedness to Dr. D. B. Marcus of Detroit and Drs. Bruce Peck, R. D. Taylor and A. C. Corcoran of the Lilly Laboratory for Clinical Research, City Hospital, Indianapolis, who supplied pertinent data of the case history and laboratory tests. Grateful acknowledgement is due also to the colleagues at the University of Chicago, Dr. Wm. E. Adams who performed the operation, Dr. E. Humphreys, who reviewed the specimen and to Drs. Milton Landowne and Barbara Spiro for special tests.

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# ANNOUNCEMENT OF THE 1948 MEETING OF THE ASSOCIA- TION FOR THE STUDY OF INTERNAL SECRETIONS

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The Thirtieth Annual Meeting of the Association for the Study of Internal Secretions will be held in the Palmer House, Chicago, Illinois, June 18 and 19, 1948.

The scientific sessions will be held in the Red Lacquer Room and registration will be on the fourth floor just outside the Red Lacquer Room. The Annual Dinner will be held in the same room on Friday, June 18th at 7 p. m. and will be preceded by a cocktail party, the location of which will be announced later. The Council will meet at 2 p. m. Thursday, June 17th.

All members of the Association who plan to attend the Thirtieth Meeting are urged to make their reservations at once with the Palmer House, stating the time of arrival and how long they plan to remain in Chicago.



# Announcement of Awards and Fellowship of the Association

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## *Nominations for Awards*

Three awards for meritorious work in endocrinology will be given at the next annual meeting of the Association. A special committee of five members of the Association chooses the recipients of these Awards, subject to ratification by the Council, and each member of the Association has the privilege of making one nomination for each award.

Nominations for the Awards should be made on special application forms which may be obtained from the Secretary, Dr. Henry H. Turner, 1200 North Walker Street, Oklahoma City 3, Oklahoma. All nominations, accompanied by a statement of the importance of the nominee's contributions to endocrinology and a bibliography of his most important papers with reprints if possible, should be sent to Dr. Turner's office not later than March 15, 1948.

### THE E. R. SQUIBB AND SONS AWARD

The E. R. Squibb and Sons Award of \$1,000.00 was established in 1939. It was given in 1940 to Dr. George W. Corner; in 1941 to Dr. Philip E. Smith; in 1942 to Dr. Fred C. Koch; in 1944 to Dr. Edward A. Doisy; in 1945 to Dr. E. C. Kendall; in 1946 to Dr. Carl G. Hartman; in 1947 to Drs. Carl F. and Gerty T. Cori. No award was made in 1943. No age or special limitation is stipulated by the donor of the award.

### THE CIBA AWARD

The Ciba Award, established in 1942, is given in recognition of the meritorious accomplishment of an investigator, not over 35 years of age, in the field of clinical or pre-clinical endocrinology. In 1944 the Award was given to Dr. E. B. Astwood; in 1945 to Dr. Jane Anne Russell; in 1946 to Dr. Martin M. Hoffman and in 1947 to Dr. Choh Hao Li. The Award is for \$1,200.00. If within two years of the date of the Award, the recipient chooses to use it to aid in working in a laboratory other than the one in which he normally is located, the Award will be increased to \$1,800.00.

### THE AYERST, McKENNA & HARRISON FELLOWSHIP

The first award of the Ayerst, McKenna & Harrison Fellowship was given to Dr. Samuel Dvoskin in 1947. The fellowship was founded in order to encourage investigation in the field of endocrinology rather than as an award



"fair" diabetic control. There was a tendency for girls in the higher levels of diabetic control to mature normally, and for those in the lower levels to be delayed in maturing. A longer-than-average growing period was generally co-existent with delayed menarche. When the onset of diabetes occurred during the years 10-14, it was likely to interfere with the maturation processes. Only  $\frac{1}{3}$  of the girls under good-to-excellent diabetic control had delayed menarche, while  $\frac{3}{4}$  of the girls under fair-to-poor diabetic control had delayed menarche. The severity of the disease added to the problem of control, since the children with less severe disease tended to maintain higher levels of control. However, the severity did not appear to be the determining factor when the disease was well controlled. The authors emphasize the importance of early and complete management in order to prevent retardation of growth and development. For the children already stunted from improper care, complete management is essential to ameliorate the retardation of growth and development.—*E.C.R. Jr.*,

MILLER, H. C. The effect of diabetic and prediabetic pregnancies on the fetus and newborn infant. *J. Pediat.* 29: 455 (1946).

It has long been recognized that the diabetic state in the mother has often been associated with many unusual phenomena in the fetus and new-born infant. Among the outstanding peculiarities of these infants, there has been noted a high mortality rate, a tendency toward an excessive birth weight and occasional anatomic changes in some of the viscera and glands of internal secretion. Until recently, it has been thought that these peculiarities of the offspring were in large part related to the maternal hyperglycemia and the manner in which it was regulated. Excessive size of the fetus was attributed directly to the fact that the fetus was maintained during pregnancy on a high carbohydrate intake. High fetal mortality was explained either by the occurrence of maternal hyperglycemia or hyperglycemia associated with an inadequate regulation of the diabetes; the high neo-natal mortality was considered to be the result of the hypoglycemia induced in the newborn infant by the overactivity of its pancreatic islet tissue. The basis for this theory rested on the observation that in some infants born to diabetic mothers an actual hypertrophy of the islet tissue could be observed. Recent studies indicate that these previously held theories are improbable. There is considerable evidence to show that all the phenomena observed in infants born to diabetic mothers are to be found among infants born before the onset of maternal diabetes. In this paper, the author reviews this newer evidence and gives statistical data concerning the incidence of fetal and neo-natal mortality, the birth weight, and the occurrence of anatomic changes in the viscera and glands of internal secretion in a series of infants born to diabetic mothers, or to mothers who subsequently became diabetic. The question is raised as to whether the increase in birth weight and the changes in the organs of these infants may be due to an increased amount of pituitary growth hormone which is not only diabetogenic for the mother but also growth-promoting for the fetus. Furthermore, the question is raised as to whether there may be a deficiency in the production of steroid hormones by the placenta during diabetes which is responsible for fetal death and for some of these alterations in the offspring of diabetic or prediabetic mothers.—*E.C.R., Jr.*



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## EFFECT OF TESTOSTERONE ON THE EXCRETION OF GLYCOGENIC CORTICOIDS

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**A**NIMAL experimentation by numerous investigators has shown that the administration of testosterone to intact rats has a definite effect on the anterior pituitary as well as on other glands of internal secretion such as the adrenal, the thyroid, the testes and the ovary (5, 6, 9, 10, 11, 12, 13, 17). In rats testosterone propionate will cause atrophy of the pituitary gland and involution of the adrenal gland; the latter occurring more readily in female rats than in males (6, 10).

Reifenstein and co-workers (8) have reported that the administration of methyl testosterone to humans will cause a decrease in the excretion of 17-ketosteroids of adrenal origin. It seemed of interest therefore to determine whether testosterone would have an inhibiting effect on other types of adrenal metabolites, particularly those associated with carbohydrate metabolism, namely the glycogenic corticoids. The present paper is concerned primarily with the effect of testosterone propionate and methyl testosterone upon the excretion of these substances. Simultaneous studies on the excretion of 17-ketosteroids were also made.

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Read before the Twenty-ninth Annual Meeting of the Association for the Study of Internal Secretions, Atlantic City, New Jersey, June 7, 1947.

## METHODS

Investigations were carried out on eight individuals: 3 normals, 2 patients with diabetes mellitus, 1 with lupus erythematosus disseminatus, and 2 with Cushing's syndrome. Complete 24-hour specimens of urine were collected on all patients before and during testosterone therapy. The urine was pooled in lots of 48-hour or 72-hour amounts and aliquots were used for the determinations. The accuracy of the collection of urine was checked by creatinine determinations. All results are reported in terms of amounts excreted per 24 hours. The glycogenic corticoids were assayed according to the bioassay method of Venning, Kazmin and Bell (15) which is dependent upon the deposition of glycogen in the livers of adrenalectomized mice. The results are expressed in terms of glycogenic units, one unit being equivalent to the activity of 1 microgram of 17-OH-11-dehydrocorticosterone. The normal excretion range for men is from 40 to 90 glycogenic units per 24 hours and for women from 25 to 70 glycogenic units per 24 hours.

The urinary 17-ketosteroids were measured by means of the colorimetric method of Holtorff and Koch (3) and a color correction was applied.

Testosterone propionate was injected intramuscularly and methyl testosterone was administered orally in the form of tablets.

## CLINICAL MATERIAL AND RESULTS

*Case 1* (Fig. 1). A woman aged 45 years, had no disease and a normal output of glycogenic corticoids and 17-ketosteroids. Measurements were made on 72-hour urine collections. In the two control periods the urinary corticoids were 42 and 45 units per 24 hours. Twenty-five mg. testosterone propionate were administered daily for 6 days. There was an initial increase in output of glycogenic corticoids which might possibly be accounted for by the fact that the individual developed a head cold at this time. However, the cold cleared up within a few days and at the end of the therapy the glycogenic corticoids had decreased to a level of 29 units per 24 hours. One week later, they had returned to their original level. As testosterone propionate is metabolized to 17-ketosteroids, the usual rise in urinary 17-ketosteroids is seen following the administration of this hormone.

*Case 2* (Fig. 1). A man, aged 55 years, had no disease, but the 17-ketosteroids in two control periods were 8.7 and 7.0 mg/24 hours—below the normal range. Determinations were made on 72-hour collections. The glycogenic corticoids were 85 and 87 units/24 hours. Forty mg. of methyl testosterone were administered orally over a period of 10 days. Then the dosage was reduced to 10 mg. for a further period of 17 days. At the end of the tenth day after 40 mg. of methyl testosterone the glycogenic corti-

coids were depressed to a level of 35 units/24 hours. On the lower dose of 10 mg. they increased somewhat to a level of 45 units/24 hours. The 17-ketosteroids had gradually decreased to a low level of 3.8 mg./24 hours by the tenth day, but increased again to 6.1 mg. when the dosage of methyl testosterone was reduced.

*Case 3* (Fig. 2). A male, aged 21 years, had no disease. Numerous assays of urinary glycogenic corticoids showed a consistently higher excretion than that of other normal males in this age group. Determinations were

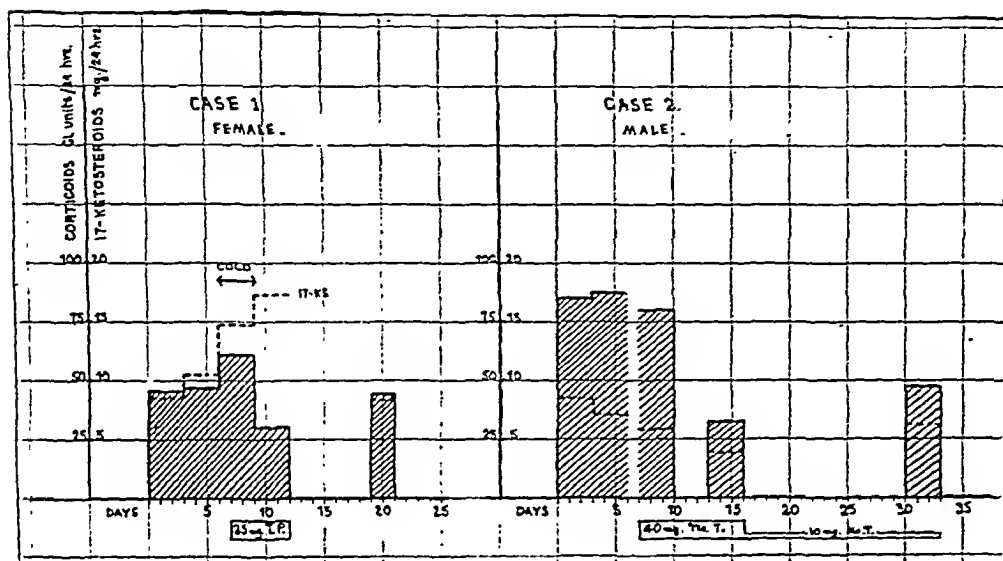


FIG. 1. Effect of testosterone propionate and methyl testosterone on the excretion of glycogenic corticoids and 17-ketosteroids in two normal individuals. The hatched columns represent the corticoids and the interrupted line the 17-ketosteroids.

made on 48-hour collections. In the two control periods, the glycogenic corticoids were 110 and 115 units/24 hours, the 17-ketosteroids 21 and 23 mg./24 hours. Fifty mg. of testosterone propionate were administered daily for a period of 6 days. Under this treatment the level of glycogenic corticoids gradually fell, reaching a low value of 40 units/24 hours.

*Case 4* (Fig. 2). A woman, aged 25 years, had diabetes mellitus. This patient was given 20 mg. of methyl testosterone orally for 5 days. Before therapy the urinary corticoids were 73 units/24 hours. With methyl testosterone the excretion rate decreased to 37 units and one week later it had risen to 83 units. The 17-ketosteroids were depressed from 9.5 mg./24 hours to 6.0 mg./24 hours. One week after cessation of therapy they were 14 mg./24 hours.

*Case 5* (Fig. 3). A young girl, aged 14 years, suffered from lupus erythematosus disseminatus. During the period of study the patient's temperature ranged from 99° to 102.4°F. Assays were made on urine collections of 48 hours. Two control periods approximately 2 weeks apart showed increased corticoid excretions of 98 and 93 units per 24 hours. The 17-

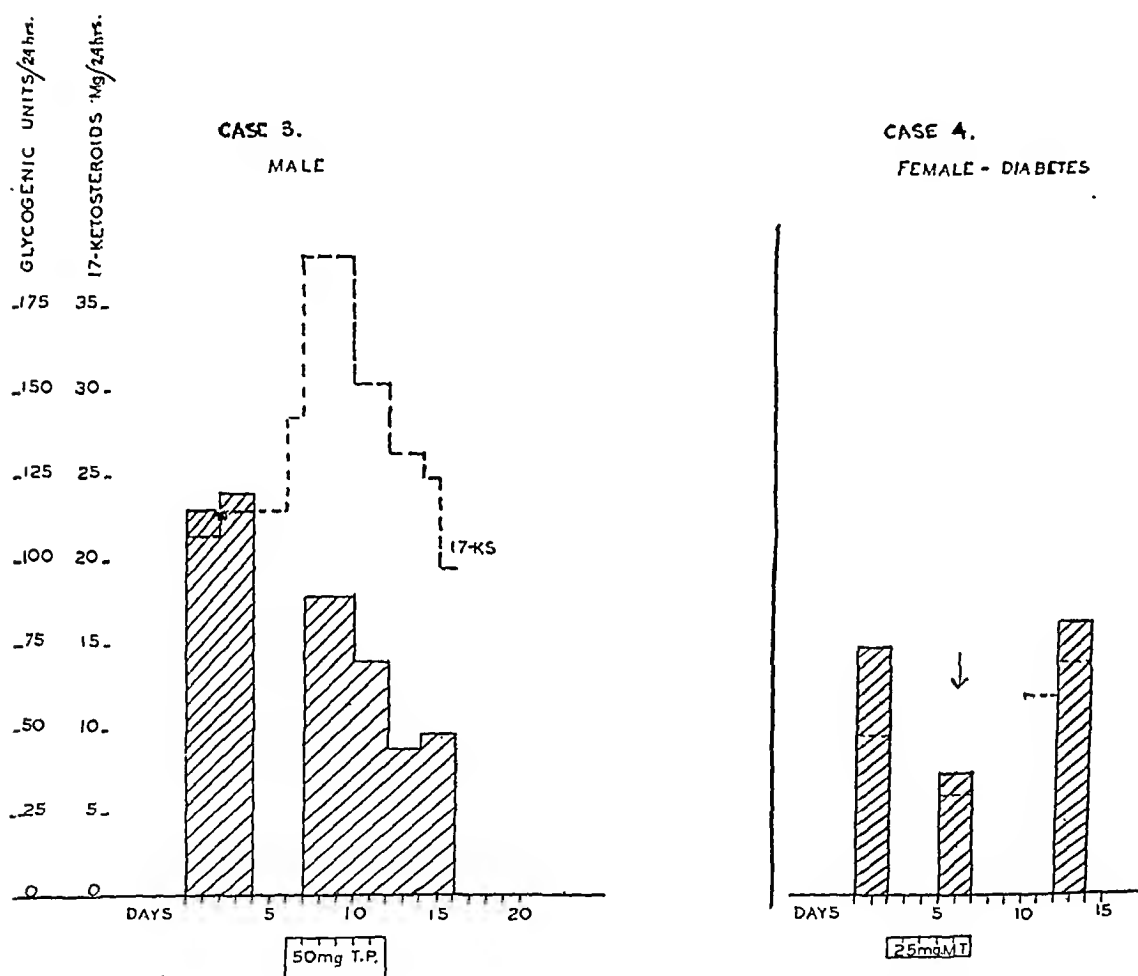


FIG. 2. Effect of testosterone propionate and methyl testosterone on the excretion of glycogenic corticoids and 17-ketosteroids.

ketosteroids averaged 4.6 and 5.5 mg./24 hours. With the daily administration of 50 mg. of testosterone propionate, the corticoids decreased to 40 units/24 hours on the fourth and fifth days, and to 29 units/24 hours on the 8th and 9th days. On the 14th and 15th days of therapy, the corticoids were 29 units/24 hours. The excretion of 17-ketosteroids rose with the testosterone propionate therapy to a high level of 21 mg./24 hours, but this was not maintained in spite of continued injections of testosterone propionate, suggesting that the endogenous supply of 17-ketosteroids was

being suppressed. There was no change in temperature or in the clinical condition of the patient during the period of study.

*Case 6* (Fig. 3). A woman, aged 40 years, had diabetes mellitus associated with hirsutism. Excretion of 17-ketosteroids and glycogenic corticoids was normal. A low blood sodium, pigmentation of the skin and hypotension suggested underfunction of the adrenal with regard to regulation of electrolyte metabolism. Three separate assays of corticoids gave values of 55,

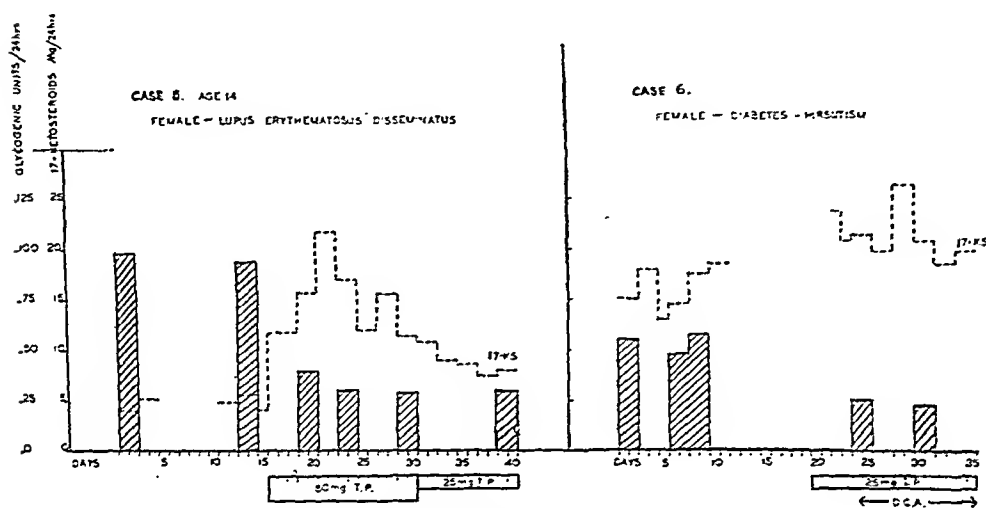


FIG. 3. Effect of testosterone propionate on the excretion of glycogenic corticoids and 17-ketosteroids.

49, and 57 glycogenic units/24 hours respectively. With the administration of 25 mg. of testosterone propionate, the corticoids were depressed to levels of 22 and 25 units. Five mg. desoxycorticosterone were administered daily beginning on the 6th day of testosterone therapy, but failed to cause any further depression of the urinary corticoids.

*Case 7* (Fig. 4). The patient was a woman, aged 42 years, with Cushing's syndrome. She was studied over a control period of 13 days. The 17-ketosteroids averaged 20 mg./24 hours and the glycogenic corticoids 260 units/24 hours. On the 14th day, 25 mg. testosterone propionate were administered daily for 13 days and then twice weekly for five weeks. The adrenals were irradiated for two weeks between days 20 and 36. On the 3rd and 4th days of administration of testosterone propionate, the corticoids decreased to 88 units. They remained at a low level throughout the treatment, varying between 55 and 70 units/24 hours. The 17-ketosteroids increased initially with the administration of testosterone propionate, but gradually fell with continued treatment until the 36th day, after which

they increased again to their original level. A glucose tolerance test made on the 3rd day showed marked impairment. When repeated on the 66th day after 7 weeks of treatment with testosterone propionate, the curve was normal. These are shown in Fig. 5.

Case 8 (Fig. 6). A man, aged 32 years, had Cushing's syndrome. Increased appetite and thirst during the past five years resulted in a striking increase in weight to 351 lbs. "Appetite rages," repeated "absences," sleep

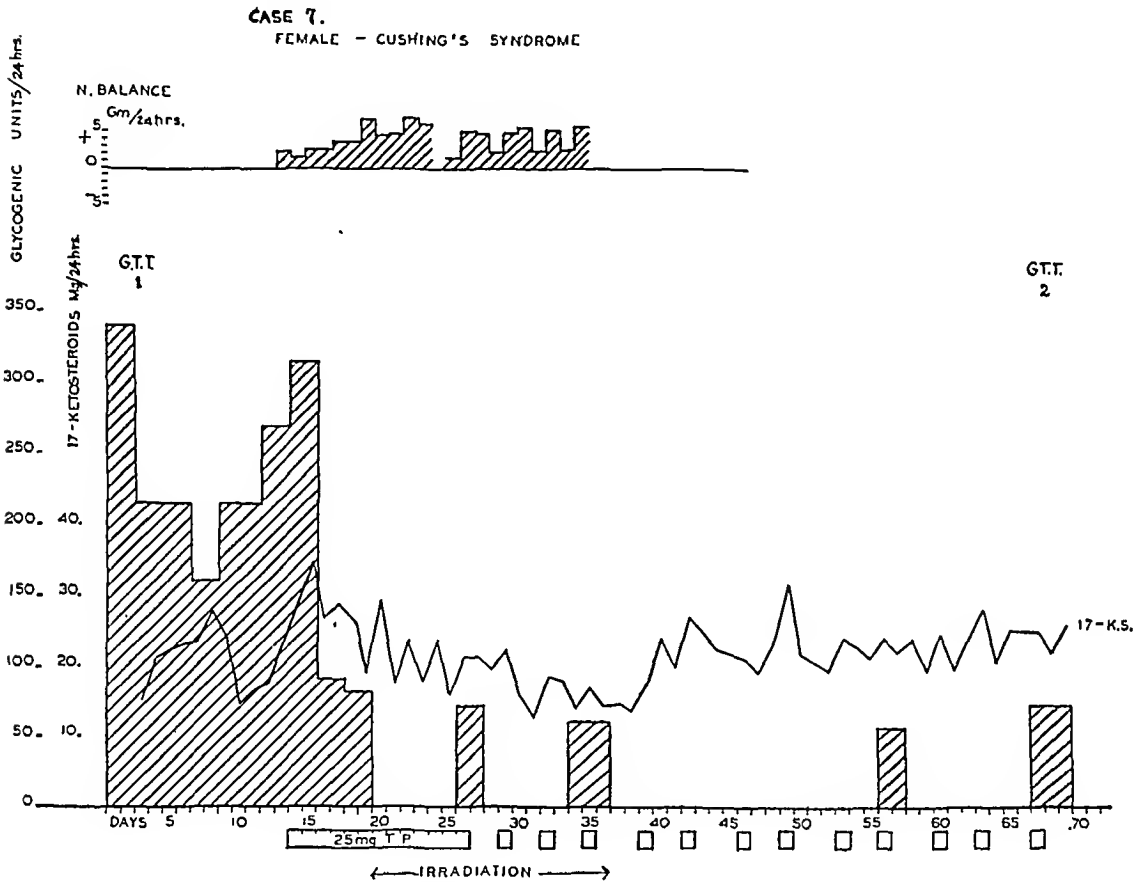


FIG. 4. Effect of testosterone propionate on the excretion of glycogenic corticoids and 17-ketosteroids in a woman with Cushing's syndrome.

disturbances, abnormal waves in basal lead of the electroencephalogram, and the result of a pneumoencephalography suggested a hypothalamic lesion. The glycogenic corticoids ranged from 356 to 546 units per 24 hours and the 17-ketosteroids from 10 to 16 mg. per 24 hours. Twenty-five mg. testosterone propionate were administered daily from the 21st day of study until the 51st day, when the dosage was increased to 50 mg. During the first 6 days of administration of testosterone propionate, on days 21 to 26, no effect on the glycogenic corticoids was seen. On days 27 to 36, the excretion of corticoids decreased to 250 units but later increased again with con-





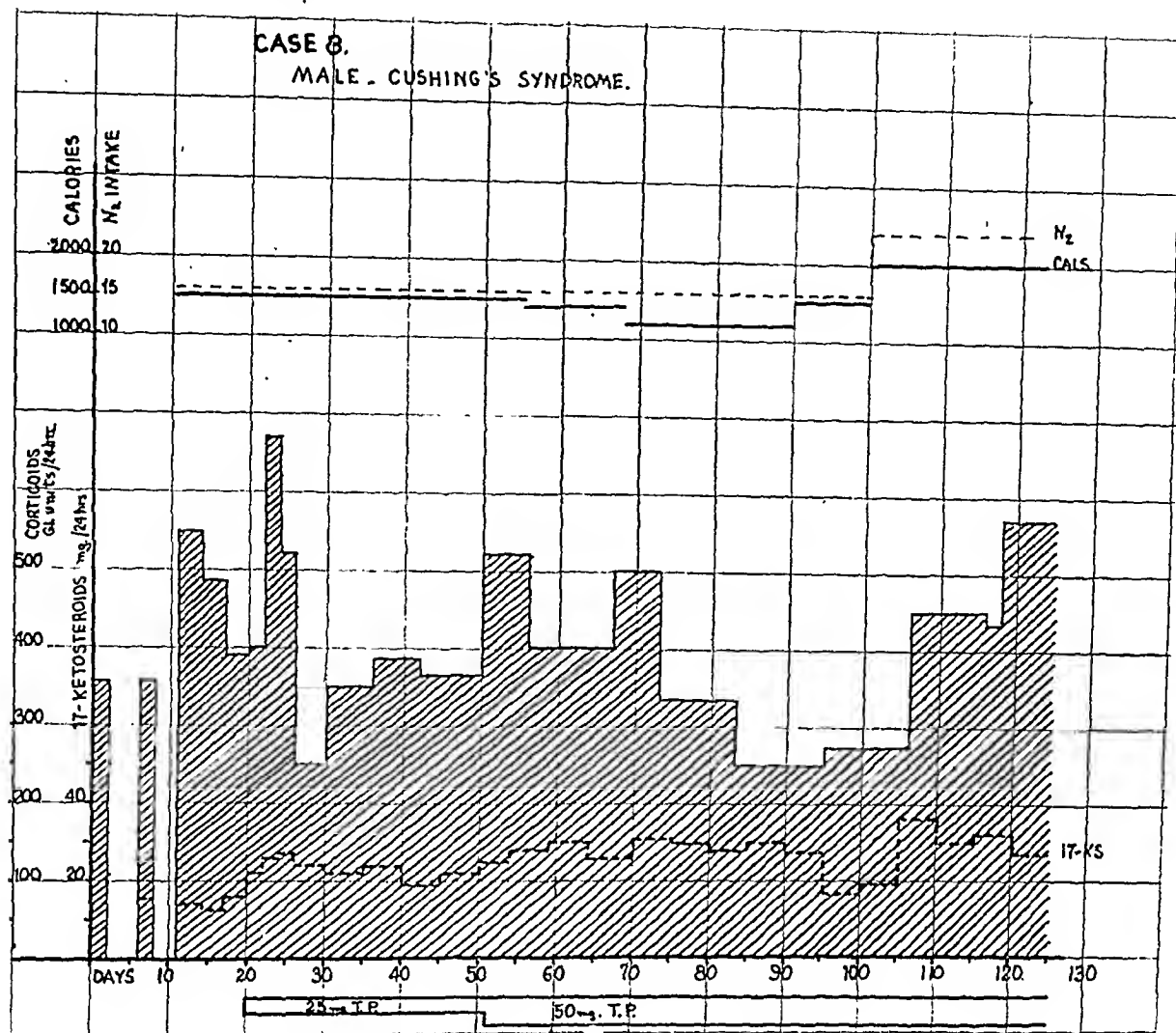


FIG. 6. Excretion of glycogenic corticoids and 17-ketosteroids in a man with Cushing's syndrome.

propionate, they decreased 60 and 75 per cent. These two patients, however, had higher control values of urinary corticoids. The patient with Cushing's syndrome (*case 7*) with a markedly increased rate of excretion of 260 units/24 hours, showed the greatest depression with 25 mg. testosterone propionate. This inhibiting effect of testosterone upon adrenal metabolism is not a permanent one as seen in *cases 1* and *4*. Within approximately a week of cessation of treatment the glycogenic corticoids and 17-ketosteroids were back to their initial levels. It is interesting to note that in *case 5* the high excretion of corticoids, probably due to a response of the adrenal to infection, was markedly reduced by testosterone therapy even though the fever persisted with temperatures ranging from 100° to 102°F.

One significant fact brought out by these studies is that with the dosages

given it has not been possible to lower the corticoid excretion much below the lower limit of normal excretion for the individual concerned, 40 units for males and 25 units for females. With regard to the effect of methyl testosterone on the excretion of 17-ketosteroids, the results in *cases 2* and *4* confirm the findings of Reifenstein and co-workers (8) that this hormone will definitely lower the excretion of 17-ketosteroids. When testosterone

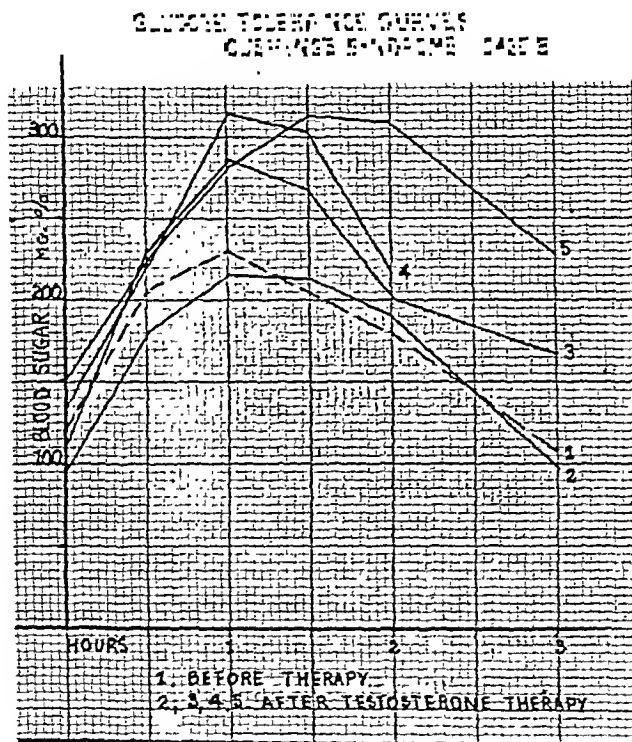


FIG. 7. Glucose tolerance curves in case 8, a man with Cushing's syndrome (1) before treatment, (2) after 31 days, (3) 72 days, (4) 127 days and (5) 214 days of testosterone therapy.

propionate is given, the added metabolites from the administered hormone, in the form of 17-ketosteroids, obscure the results. However, it is observed that in many of the cases with continued administration of testosterone propionate the level of 17-ketosteroids tends to fall, thus suggesting that those derived from endogenous sources are being depressed.

It was found that in case 7, a woman with Cushing's syndrome, it was possible to maintain the glyco-genic corticoid excretion within normal limits

with administrations of 25 mg. testosterone propionate twice weekly. The changes that occurred in the glucose tolerance curves were most significant. Before therapy, at a time when the glycogenic corticoid excretion was high, average value of 260 units/24 hours, the test showed an impaired tolerance curve; after 5 weeks of therapy with testosterone propionate, both glucose tolerance and glycogenic corticoid excretion were normal.

In contrast to this we find that in *case 8*, a male, also with Cushing's syndrome, failed to show any continued effect of testosterone therapy. There was a decline in the corticoid excretion from 670 units to 250 units over a period of 10 days following the daily administration of 25 mg. of testosterone propionate, but this was not maintained and the excretion rose again. When the testosterone propionate was increased to 50 mg. no effect was observed. The glucose tolerance curves showed increasing impairment in function throughout the study, and their character was not altered by testosterone therapy (Fig. 7).

There appears to be little in the physical and clinical histories of these two patients with Cushing's syndrome to suggest why one should respond to testosterone therapy and the other one fail to do so. The onset of the disease in *case 8* had been more rapid and much more severe than in *case 7*, and it may be possible that the pathological lesion responsible for the disease in *case 8* was more difficult to influence than that in *case 7*. In earlier work regarding the effect of testosterone on adrenal atrophy, it was observed that the response was more readily obtained in female than in male rats (6, 10). However, in the two studies on male individuals with no disease, *cases 2* and *3*, it was possible to show that both testosterone propionate and methyl testosterone were effective in causing a reduction in excretion of adrenal metabolites. The three patients with Cushing's syndrome, successfully treated with testosterone propionate as reported by Albright et al. (1), were all women as was the patient reported by Perloff et al. (7), who noted an improvement in the tolerance for dextrose after prolonged testosterone therapy. The patient of Deakins et al. (2), a 15 year old girl with Cushing's syndrome, failed to show demonstrable clinical improvement with testosterone propionate and methyl testosterone despite the definite effect which these hormones were shown to have had upon her nitrogen balance. Whitelaw (16) reported that a 17 year old boy with the same disease, under testosterone propionate therapy showed no improvement in the osteoporosis or tolerance for glucose although there was a marked gain in strength and weight and an increased excretion of creatine.

What is the mechanism of the action of testosterone upon adrenal metabolism? Is this inhibiting effect a direct action of testosterone itself upon the adrenal or is it a result of the suppression of those tropic hormones of the anterior pituitary which control adrenal metabolism?

Proof that this effect is mediated through the pituitary has been supplied by the work of Selye (10) who has shown that in hypophysectomized rats maintained on adrenotropic hormone, testosterone fails to cause atrophy of the adrenals. Reifenstein and co-workers (8) have put forth evidence to support the theory that the tropic hormone responsible for the production of 17-ketosteroids from both the testis and the adrenal is the luteinizing hormone. The more recent work of Thorn, Prunty, and Forsham (14) and of Mason and co-workers (4), however, on the effects of purified adrenotropic hormone reveals that this pituitary hormone will also stimulate the production of 17-ketosteroids as well as 11-oxysteroids. An interference with the production of adrenotropic hormone alone by the administration of testosterone could, therefore, equally well account for the reduction in excretion of both glycogenic corticoids and 17-ketosteroids.

The beneficial effect of testosterone propionate therapy in Cushing's syndrome may therefore be due not only to the metabolic action of testosterone directly opposing the effect of the glycogenic type of corticoid on protein and carbohydrate metabolism as suggested by Albright but also in certain cases to a repression of the over-activity of the adrenal cortex.

#### SUMMARY

The excretion of urinary glycogenic corticoids and 17-ketosteroids was followed in a series of 8 individuals before and after the administration of either testosterone propionate or methyl testosterone. These included 3 normals, one woman and 2 men; 2 women with diabetes; one young girl with lupus erythematosus disseminatus; and 2 cases of Cushing's syndrome, one male and one female.

In all the cases, with the exception of the male with Cushing's syndrome, testosterone therapy caused a reduction in the rate of excretion of glycogenic corticoids.

In the cases under methyl testosterone therapy a decrease in urinary 17-ketosteroids was also noted. It is suggested that this inhibition caused by testosterone therapy is mediated through the pituitary by the reduction in production of adrenotropic hormone.

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# TRUE HERMAPHRODITISM

## ENDOCRINE STUDIES IN A CASE OF OVOTESTIS

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THE occurrence of both male and female gonads in one person is of sufficient rarity to invite thorough study of such a case. Pseudohermaphroditism is not uncommon, but the occurrence in one person of one ovary and testis, or an ovotestis, proved histologically, has been reported only 40 times in the literature (1, 2, 4, 5, 6, 7). Excellent reviews of true hermaphroditism have been written by McIver and associates (4) and Young (7). In both of these reports the predominant abnormality was the presence of ovotestes, either unilateral or bilateral. Only seven true ovaries were found in the series, and six true testes; the remainder (except for four gonads of undetermined type) were ovotestes. The clinical features of these cases were such as to cause suspicion of hermaphroditism with insufficient development of the attributes of either sex. The rarity of this condition warrants the report of such a case in detail.

### REPORT OF CASE

D. M. (T-46-226172), a negro "female," aged 36, was admitted to Charity Hospital in New Orleans on July 18, 1946, because of a "mass between her legs," which had been present as long as she could remember, but which had recently increased so much in size as to cause local discomfort.

The patient was one of twins and was considered a female at birth. From the history, it would seem that at birth a small lump was present in the perineum beneath which was the urethral orifice. Apparently, the patient's mother had no reason to suspect an abnormality and the child was reared as a sister to the other girls.

Adolescence began between the tenth and twelfth years. Development followed the female pattern, with mammary glands enlarging to a degree comparable to the patient's twin sister. Mammary development continued until the twentieth year, when slow regression began. "Menstruation" began at the age of 11 years, with regular cycles, at 28 to 30 day intervals. The flow was said to be scanty and lasted one and a half days. The site from which the flow emerged was unknown to the patient, who was unconcerned about the anatomic abnormalities. Mild abdominal cramps accompanied the menses for about five years after the menarche. Axillary and pubic hair developed in feminine fashion with little or no overgrowth in the earlier years. From the twentieth year, facial

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hair on the upper lip and chin began to appear, and some increase was noted along the linea nigra of the abdomen. There was no vocal change and at present the patient sings soprano in a choir. As the patient grew, the mass enlarged becoming more prominent on the right side until its present size prompted her to seek medical advice.



FIG. 1. Photograph of patient showing general body development.

The patient's thoughts and actions were always mediated by the conviction that she was a female. She preferred the company of men and had several beaux. Marriage was contemplated at one time, but her fiancé was accidentally killed. Although she had frequent dates, she denied any attempts at sexual activity.

The patient had four brothers and two sisters all of whom were normally developed; the two sisters were married, and one was the mother of two normal children.

Examination revealed a well nourished negro "woman" weighing 112 pounds, 65 $\frac{1}{4}$  inches tall, with a span of 68 inches. Facial hair with masculine distribution was sparse but conspicuous. The breasts were well developed but atrophic changes were evident. A large inguinal hernia on the right side with considerable chronic edema of the sac protruded about 10 to 12 cm. from the level of the pubic tubercle; it was not completely reducible (Fig. 1).



FIG. 2. Photograph of patient's external genitals.

The external genitals were neither masculine nor feminine. There was a large phallus, measuring approximately 3 cm. in length (Fig. 2). Its prepuce disappeared into the pubic skin without forming any rudimentary labia minora. No labia majora could be identified and there was no scrotal swelling. The urethral orifice was situated about 1 cm. dorsal to the base of the phallic structure. Rectal examination revealed a granular tag which bled easily upon trauma. No rudimentary structures comparable to a prostate gland or müllerian system could be felt on palpating the anterior rectal wall. Cystoscopic examination revealed a normal female urethra without a vaginal orifice or a verumontanum.

A plain roentgenogram of the kidney, ureter and bladder showed essentially normal structures. Roentgenologic examination of the skull disclosed indistinct posterior clinoid processes but no other abnormalities.

Preoperatively, urinary gonad-stimulating hormone assays showed  $>96$   $<192$  m.u./24 hrs., and  $>52.8$   $<100$  m.u./24 hrs.; 17-ketosteroid determinations were 7.6



and 7.5 mg./24 hrs. The urinary gonad-stimulating hormones were estimated by the uterine weight method of Klinefelter, Albright and Griswold (3) and the 17-ketosteroids by a modification of the Zimmerman reaction with dehydro-iso-androsterone as a standard.

On July 26, 1946, under spinal anesthesia, the right inguinal hernia was repaired. The sac contained omentum incarcerated with a sliding hernia of pelvic organs. There was a rudimentary uterus with a well developed left fallopian tube and a left gonad which appeared grossly to be a small ovary. A nodular eminence on one pole of the ovary was removed for study. The ovary was bisected and a slice of tissue was taken also for study. The right tube extended into the herniated mass and disappeared into the sac wall. No



FIG. 3. Photomicrograph of section of ovotestis (ovarian portion) with normal ovarian stroma but absence of primordial and growing follicles.

fimbriated end was noted and the round ligament was indistinct. At a short distance from the point of disappearance of the right tube a nodule, 2.5 by 1 cm., was palpable in the sac; this nodule did not have a free border and did not simulate the right gonad grossly. No gonad was seen. The omentum was free, and the redundant sac, with the nodule, was severed from the rudimentary uterus. The hernia was repaired, and the granular anal tag was also removed. It was felt that this might contain aberrant endometrial tissue from which cyclic bleeding occurred.

On August 10, 1946, a plastic operation was performed. The redundant skin was used to form a vagina behind the urethral orifice. Subsequently, the greater portion of this whole thickness graft sloughed away. The vagina was maintained with an obturator for a time but has since contracted to a mere sinus tract. A further plastic operation will be needed.

The postoperative course was entirely uneventful. Five weeks after the operation 17-ketosteroids were determined on three occasions and, the amounts were 5 mg./24 hrs., 4.9 mg./24 hrs., and 5.6 mg./24 hrs. The urinary gonad-stimulating hormone was assayed in two of these 24-hour urine collections, both assays containing  $>96 <192$  m.u./24 hrs. After nine months, the patient is still in good health and further "menstruation" has not occurred.

Histologic examination of the hernial sac revealed an ovotestis protruding from the lining. The larger portion of the gonad was well differentiated ovarian stroma, with several large corpora albicantia (Fig. 3). No growing follicles or ova were observed in the sections available, but there was a Walthard cell rest close to the surface in one section.

The testicular portion of the gonad was located on the deeper surface, corresponding to the medullary border. There were definite testicular tubules, well differentiated in outline, but in varying degrees of degeneration. Hyalinization and fibrosis were rather marked. No evidence of spermatogenesis could be seen (Fig. 4a). Interposed between

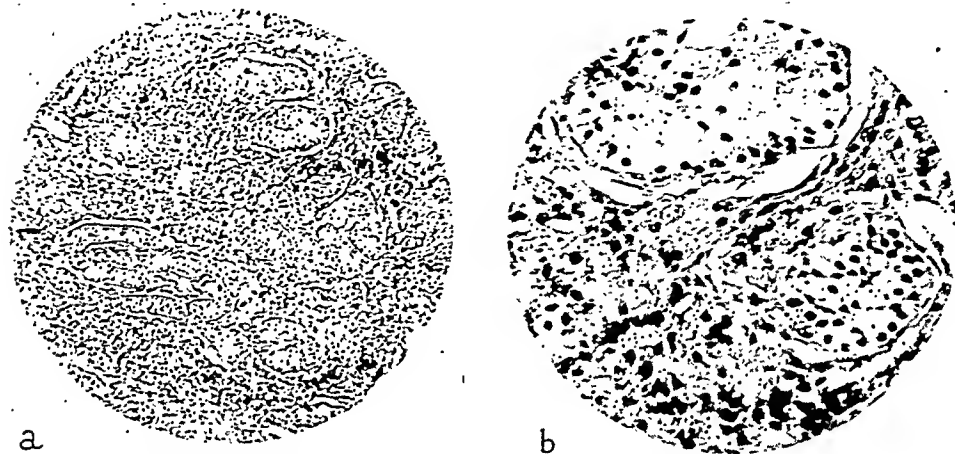


FIG. 4(a). Low power magnification of ovotestis, showing numerous testicular tubules in all stages of degeneration. Spermatogenesis is absent, and hyalinization of many tubules can be seen. Small clumps of interstitial cells are numerous. (b). High power magnification of a tubule showing interstitial cells wedged in between.

tubules, and often in large clumps, were masses of interstitial cells of Leydig (Fig. 4b). A well formed rete testis was present (Fig. 5).

Extending from one pole of the ovotestis was a thickening of the hernial sac which resembled, histologically, the spermatic cord or epididymis, although the differentiation was immature (Fig. 6). A neighboring strand of tissue consisted of fibromuscular tissue, lined with columnar epithelium, which resembled tubal epithelium.

The biopsies of the left ovary consisted of a small portion of normal ovarian stroma, also with corpora albicantia, but no growing follicles or ova. The nodular portion contained a small Brenner tumor surrounded by fibrous stroma showing little hyalinization (Fig. 7).

Examination of the rectal polyp revealed only rectal glands surmounted by stratified squamous epithelium.

#### COMMENT

This case is one of true hermaphroditism, with a normal ovary on the left and an ovotestis on the right. Of the 40 cases reported in the literature, only 11 were of the lateral or alternating type, with an ovary on one side

and a testicle on the other. Eleven patients had bilateral ovotestes, 7 had ovotestes combined with testes and 7 had an ovary with the ovotestis. Four patients had ovotestes with the other gonad unidentified.

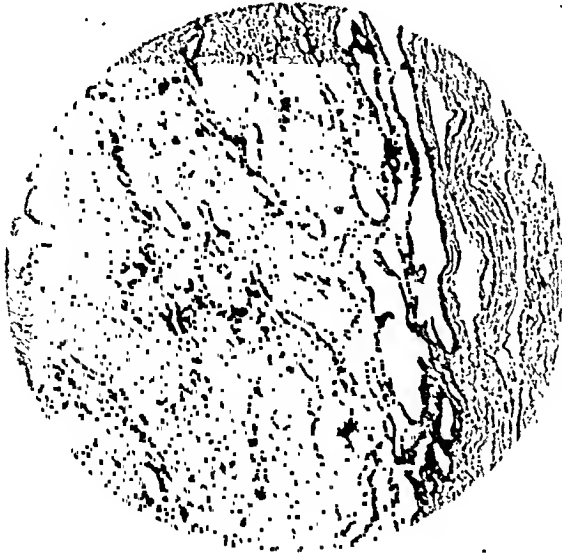


FIG. 5. Low power magnification of section of ovotestis showing well developed rete testis.

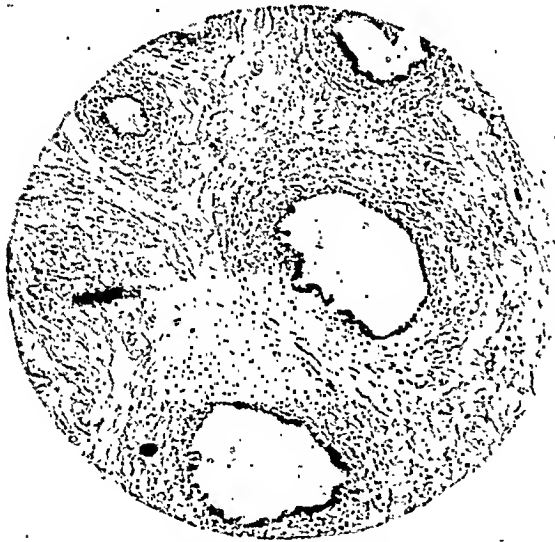


FIG. 6. Low power magnification of section of hernial sac containing fibromuscular tissue, lined with columnar epithelium resembling the epididymis.

The frequency of inguinal hernia in the reported cases (14 of 40 cases) is striking. When associated with any sexual abnormality, inguinal hernia should immediately suggest the presence of intersexuality of some degree.

The principal complaint of this patient was the hernia, not the intersexual status.

Hormonal assays have been performed in only a few of the cases reported. In our case, the 17-ketosteroid excretion was somewhat lower than anticipated. In our laboratory, the range of 17-ketosteroids in the normal female extends from 4.3 to 18.2 mg./24 hrs. with a mean of 8.7 mg./24 hrs. The range for normal males is from 7.2 mg./24 hrs. to 27.2 mg./24 hrs. with a mean of 15.9 mg./24 hrs. The amount excreted in our case is, therefore, among the lower values for the female. The decrease in values

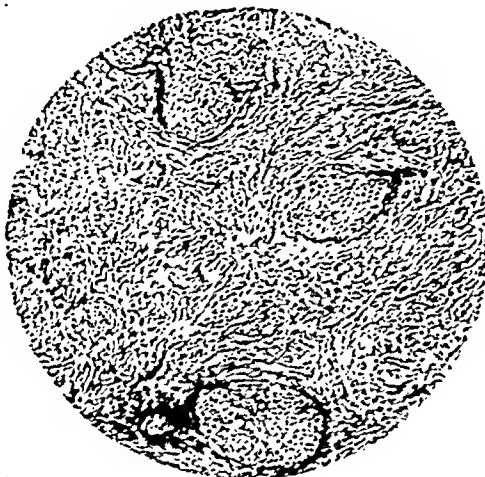


Fig. 7. Low power magnification of section of nodule of left ovary, with small Brenner tumor, consisting of well defined epithelial elements surrounded by fibrous stroma.

obtained postoperatively suggested that a portion of the excreted 17-ketosteroid was derived from the testicular tissue, which was removed, although spontaneous fluctuations in excretion are not uncommon.

We have not seen a normal male or female in whom the urinary gonad-stimulating hormone excretion was as great as the 96 m.u./24 hrs. which this patient showed. It is of interest to note that although the 17-ketosteroid excretion dropped after operation, indicating a loss of hormonally active gonad, this was not borne out by a concomitant rise in urinary gonad stimulating hormone. The relatively high urinary gonadotropic hormone values suggested that gonadal failure existed. The inactivity of the ovarian sections was confirmatory.

#### SUMMARY

1. A case of true hermaphroditism with an ovotestis on the right and an ovary on the left is presented.

2. Manifestations of normal female sexual development with superimposed facial hirsutism and an enlarged clitoris were distinguishing features.

3. The ovotestis was found in a hernial sac as in 14 other cases in the literature.

4. The 17-ketosteroid excretion was reduced postoperatively; this suggests bio-activity in the testicular portion.

5. The gonadotropic excretion in the urine was elevated indicating gonadal failure.

#### ACKNOWLEDGMENT

The authors wish to express their sincere appreciation to Dr. William Sternberg for his assistance in the pathologic studies and in preparation of the photomicrographs.

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# A COMPARATIVE STUDY OF VAGINAL AND CERVICAL CORNIFICATION IN HUMAN SUBJECTS\*

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THE advent of the cervical os smear (1) for the diagnosis of uterine cancer has led to extensive comparative studies of the vaginal and cervical methods in this laboratory. Cervical smears have been found to contain a larger concentration of cancer cells in positive cases. It has also been observed that almost invariably a greater concentration of the estrogenic cornified cells is found in the cervical smear than in the vaginal smear. This observation has prompted a study of the cornification counts of smears from the cervix compared with those from the vagina in 125 patients.

The cases were chosen at random from our files of patients who had had both vaginal and cervical smears taken at the same time. Patients with cancer and those patients who showed total absence of estrogen, e.g., normal senile patients or castrates, were excluded. The smears covered all stages of the menstrual cycle and a large variety of age groups. It was found that 88.8 per cent of the cases showed the cervical cornification count to be higher than the vaginal cornification count, varying from 5 per cent to 55 per cent. Equal cornification levels were observed in 7.2 per cent and 4 per cent showed a higher count in the vaginal than in the cervical smear. (This varied from 5 per cent to 10 per cent.) The over-all average difference between the cervical and vaginal cornification counts was 15.5 per cent.

## METHOD

A speculum was used in taking both the vaginal and cervical smears. Selective smears and scrapings were used in preference to a random aspiration. An area of the lateral wall of the vagina was lightly scraped with a wooden spatula and the scrapings were spread over a glass slide. Cervical scrapings were taken using the cervical spatula designed to scrape the squamous margin of the squamo-columnar junction, according to the technique described by one of us (2). The slides were stained with Papanicolaou's polychrome stain.

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Cornification levels are estimated by counting the number of cornified cells per hundred epithelial cells of the squamous type. The result is expressed in percentage and referred to as the cornification count or cornification level. A cornified cell is recognized as much by its morphological as by its staining characteristics. It is defined as a large, flat, wafer-like epithelial cell with a small round or oval pyknotic nucleus. The cytoplasm is acidophilic and therefore shows a pink coloration with the polychrome stain of Papanicolaou. The "pre-cornified" epithelial cells are morphologically similar, but have a basophilic cytoplasm which stains blue-green. Other immature squamous cell-types also take the basophilic stain.

It is important to eliminate cells showing "pseudo-cornification" where an acidophilic staining reaction occurs in a cell showing nuclear morphology of immaturity. Such cells are not included in the cornification count.

It has been shown that the cornification count varies in individual patients with the physiological changes during the menstrual cycle (4, 5). The cornification level rises gradually during the follicular phase, paralleling the production of estrogen by the ovarian follicle and reaches a peak, usually over 50 per cent, at ovulation. The count then drops irregularly during the luteal phase of the cycle to a premenstrual low, approaching zero.

It has been shown that when estrogen is given orally or by injection to post-menopausal and castrate women the cornification level rises from a normal zero to levels seen in normal ovulatory cycles (5). This rise is evident after only a few days of therapy and is directly proportional to the dosage of estrogen administered. Moreover, if these women are given estrogen in the form of vaginal suppositories, the time lag between instigation of therapy and high cornification levels is dramatically lessened. This has been observed in our laboratories.

It is suggested that the variability in vaginal and cervical cornification counts may indicate either:

1. A greater sensitivity of the cervical epithelium to estrogen.
2. A difference in local concentrations of estrogen with a higher concentration in the cervix.
3. A combination of (1) and (2).

There are a number of observations which suggest that the cervix is particularly sensitive to estrogens. When müllerian tract carcinoma is produced in experimental animals by large doses of estrogens administered over a long period it is usually localized to the cervical tissue, suggesting that this tissue is the most highly reactive of all of the tissues of müllerian duct origin. Scannon described prenatal growth and natal involution of the human uterus and shows that after the 20th week in utero the uterus responds to the growth-stimulating effects of estrogens by enlarging. While

the body enlarges to only a limited extent, the cervix becomes twice as large in relation to the body of the uterus as it had been before. After birth, when presumably the estrogen stimulus is withdrawn, the cervix becomes relatively smaller in relation to the body of the uterus.

That the higher concentration of cornified cells in the cervix may be related to inflammation is suggested by the work of Brunelli (3). He re-

## DIFFERENCE IN VAGINAL & CERVICAL CORNIFICATION COUNTS

(ENDOGENOUS ESTROGEN)

(125 CASES)

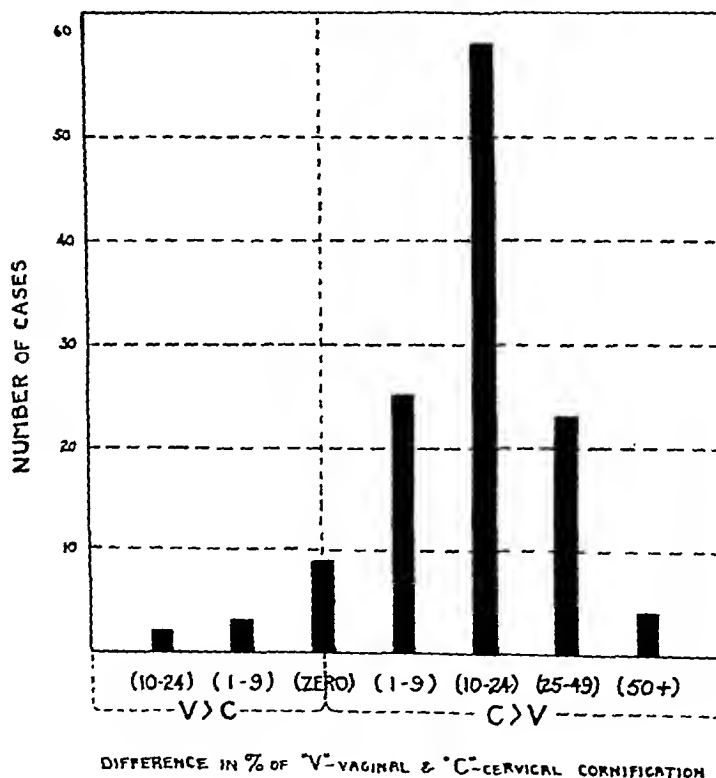


FIG. 1

ported the concentration and fixation of estrogen in inflamed tissues in rabbits.

The great frequency of chronic cervicitis in humans (80 per cent) arouses speculation as to whether there may be some etiological relationship between this disorder and the findings herein reported.



## CONCLUSIONS

1. In a study of vaginal and cervical cytology smears on 125 female patients of all ages, a persistently higher cornification index was found in the cervical than in the vaginal smear. Factors which may be responsible for this variability are discussed.

2. If the cornification count is to be used as a quantitative index of the presence of endogenous estrogens in the human subject, a consistent technique for preparation of smears is important. Selective smears or scrapings with a spatula from a selected area of the vagina or cervix should give more accurate results than a random aspiration with a pipette.

## ACKNOWLEDGMENT

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# THE USE OF HYPERTONIC SALINE INFUSIONS IN THE DIFFERENTIAL DIAGNOSIS OF DIABETES INSIPIDUS AND PSYCHOGENIC POLYDIPSIA

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**O**BJECTIVE methods for differentiating polyuria and polydipsia of neurohypophysial origin from psychogenic polydipsia have hitherto been limited to prolonged urine concentration tests (2), a difficult procedure to carry out in many instances because of the attendant discomfort and lack of cooperation on the part of the patient.

Gilman and Goodman (4, 5) found that dehydration, as well as the oral administration of hypertonic saline, led to the appearance of an antidiuretic substance in the urine of normal, but not of hypophysectomized rats; an observation which has been confirmed in several other species of experimental animals (1, 7, 9, 11, 12, 14, 15, 16, 17).

Hare and his associates in a series of studies on normal dogs (8) have shown that the concentration of chlorides in the tubular reabsorbate (R) or the ratio of R to plasma chlorides (R/P) and the urine flow, are all decreased following infusions of hypertonic saline, concomitantly with an increase of antidiuretic substance in the urine. In hypophysectomized dogs, on the other hand, the procedure was associated with an elevation of chloride R/P, an increased urine flow and the absence of antidiuretic substance in the urine. These investigators (3) have also shown that these phenomena were due not to the sodium or chloride ion per se, but to a non-specific increase in the osmotic pressure of the plasma; the administration of hypertonic solutions of urea, sodium sulfate and sodium chloride to normal dogs was equally as effective in inducing the release of antidiuretic substance in the urine and the formation of a tubular reabsorbate with a lower osmotic pressure than that of the plasma. In 1944 (10) their studies were extended to man, their subjects consisting of one normal male and three patients with polyuria and polydipsia. On the basis of urine flow, the concentration of chloride in the tubular reabsorbate and the titer of antidiuretic substance in the urine during the administration of intravenous hypertonic

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saline, they were able to differentiate between psychogenic polyuria and polyuria due to neurohypophyseal lesions.

Since this differentiation is so often difficult to achieve by other methods, it seemed desirable to investigate the reliability of this new diagnostic procedure by a study of a larger group of normal subjects and those with polyuria and polydipsia.

#### METHOD OF STUDY

Normal subjects and patients with polyuria and polydipsia were studied.<sup>1</sup> Their case histories are abstracted in the appendix. All antidiuretic therapy was stopped prior to the test for a period sufficient to permit the reappearance of polyuria and polydipsia, preferably to its original magnitude. Fluids were withheld for eight hours preceding the test but food was permitted. The subjects were hydrated with water, twenty cc. per kilogram by mouth, over a period of one hour. Thirty minutes after the period of hydration was begun, an indwelling catheter was inserted. Urine specimens were collected in fifteen minute periods and urine flow calculated in cc. per minute. After two control periods with an adequate urine flow, i.e., greater than five cc. per minute, an infusion of 2.5 per cent sodium chloride was begun. The hypertonic saline was administered intravenously at the rate of 0.25 cc. per kilogram per minute for forty-five minutes. If no decrease in urine flow occurred during the infusion or in the first two post-infusion periods (15 minutes each), 0.1 unit of pitressin (Parke-Davis) was given intravenously and its effect on urine flow observed.

#### RESULTS

Nine healthy young adults, 8 males and 1 female, showed a prompt and marked decrease in urine flow during the infusion or the first period thereafter (Table 1 and Fig. 1), the flow being reduced to 7-26 per cent of the maximum during the pre-infusion periods. In 3 instances the maximal urine flow occurred during the first 15-minute period of the infusion. That the reduction of urine flow was not due to saline per se was evident from the failure of isotonic saline infusions to inhibit urine flow (Fig. 2). The reduction of urine flow was not of spontaneous origin, diuresis continuing after hydration alone for periods up to one and a half hours, the usual duration of the test (Fig. 2). In 2 of 3 normal subjects, the decreased urine flow during the infusion could be abolished (Fig. 2) or delayed (Fig. 1, subject T. C.) by previous overhydration or the continued ingestion of water during the infusion.

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<sup>1</sup> We are indebted to Dr. Henry Barnett of the Department of Pediatrics, Cornell University Medical College, for permission to include his results in two cases (*L. G. and N. B.*) studied on the Pediatrics Service.

## NORMAL SUBJECTS

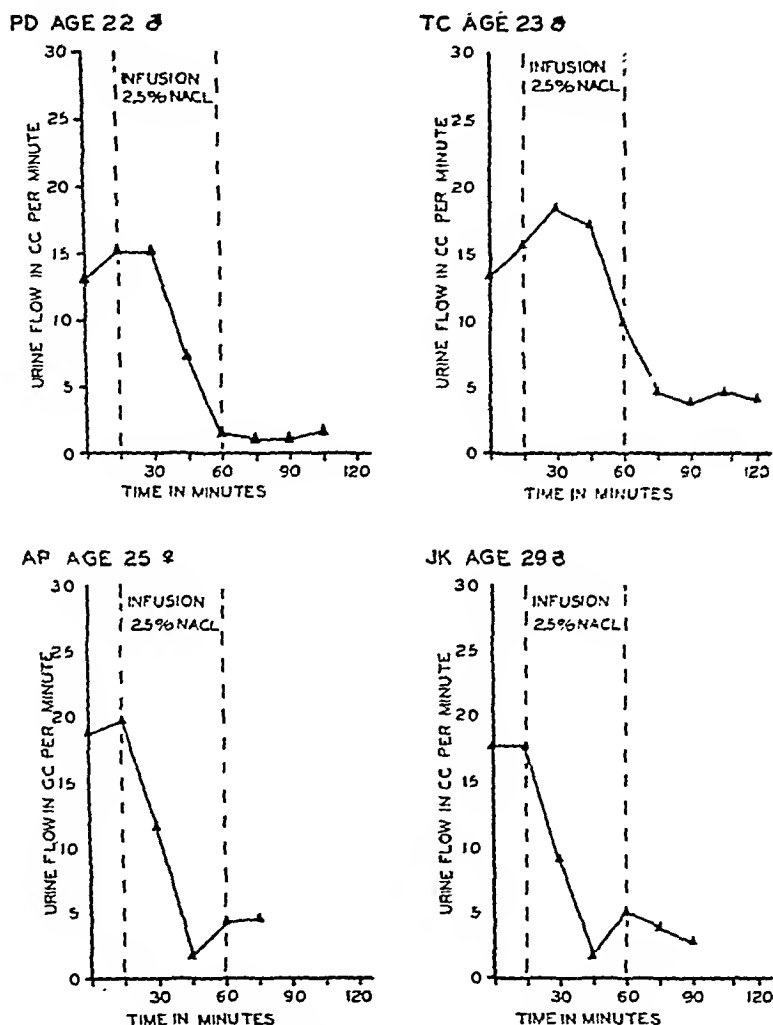
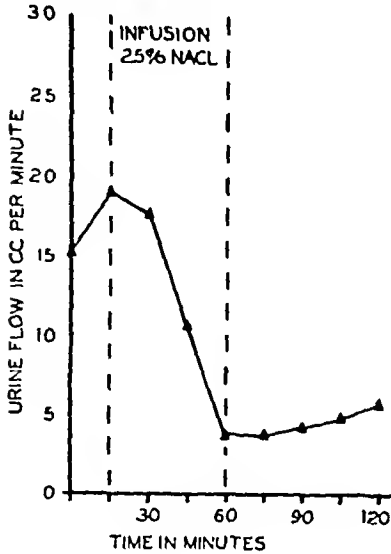


FIG. 1. Response of 4 representative normal subjects to infusion of 2.5 per cent NaCl (0.25 cc/kg/min. for 45 minutes) following previous hydration. Subject *T.C.*, maximal urine flow occurred during the first 15-minute period of the infusion and there was a slight delay in the antidiuretic response which was, however, maximal in the first post-infusion period (see text).

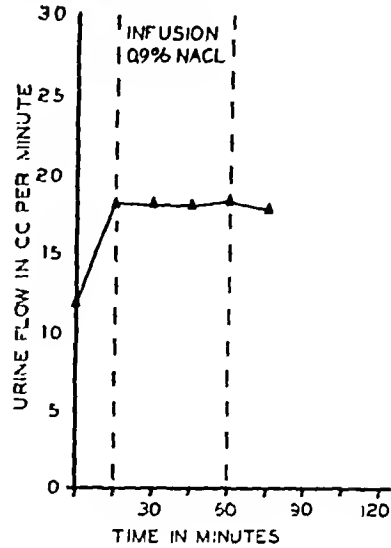
Investigations were made on the possible influence of renal disease on the reliability of this test procedure. Two patients with moderate renal insufficiency, one with renal calculi, the other with renal calcinosis, and one patient with renal calculi and minimal renal damage, all showed a prompt

## NORMAL CONTROL

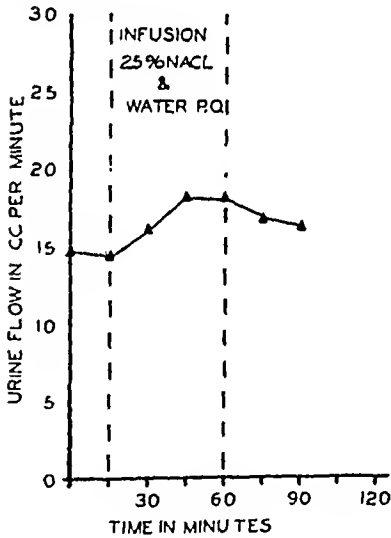
JR AGE 24 ♂



JR AGE 24 ♂



JR AGE 24 ♂



JR AGE 24 ♂

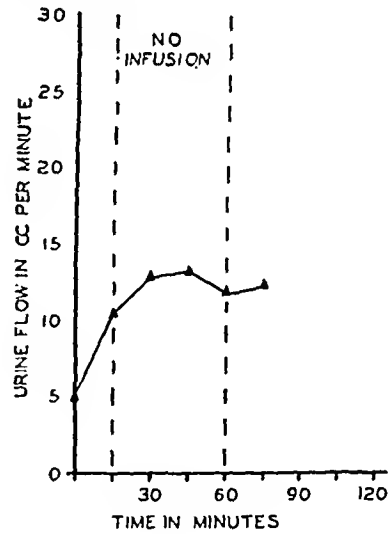


FIG. 2. Illustrates characteristic antidiuretic response of normal subject to hypertonic NaCl infusion (upper left) and failure of isotonic NaCl infusion to induce antidiuresis (upper right). Lower left graph illustrates the abolition of antidiuresis by continued oral hydration during the infusion of hypertonic saline. Lower right graph shows the sustained diuresis following hydration in the absence of a hypertonic saline infusion (see text).

reduction in urine flow comparable to the normal subjects during the infusion of hypertonic saline (Table 1). In 3 patients with severe renal insufficiency the urine flow during the period of hydration was so low the test could not be carried out.

Eleven patients with polyuria and polydipsia without glycosuria were studied. In contrast to the response of normal subjects, 8 showed a continued diuresis during and after the infusion with a prompt reduction of urine flow following the administration of pitressin (Table 1, Fig. 3). These

## DIABETES INSIPIDUS

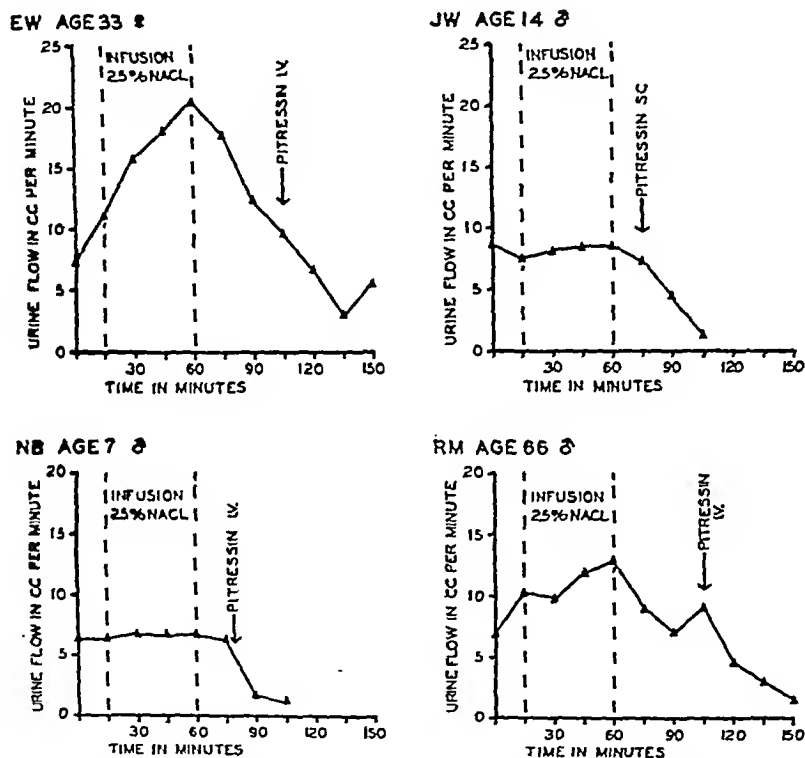


FIG. 3. Four representative responses of patients with diabetes insipidus of neurohypophysial origin. Note continued diuresis and prompt response to intravenous pitressin. In *E.W.* the reduction of urine flow in the post-infusion period prior to pitressin was only 13.6 per cent below the pre-infusion maximum.

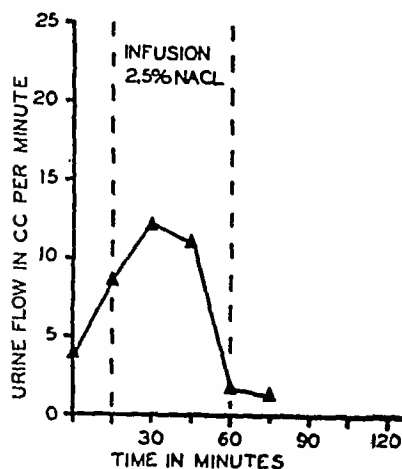
results provided objective support to the clinical impression that these patients had diabetes insipidus due to insufficiency of the neurohypophysis.

Three of the subjects with polyuria and polydipsia showed the marked decrease in urine flow characteristic of the normal subject, during or immediately after the infusion of hypertonic saline (Fig. 4). The psychogenic origin of their polydipsia and polyuria was confirmed by their subsequent freedom from symptoms without replacement therapy.

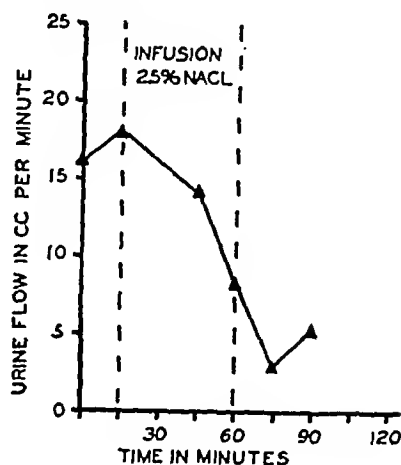
A comparison of representative responses of a normal subject and of

## PSYCHOGENIC POLYDIPSIA

RH AGE 46 ♀



EK AGE 19 ♀



LG AGE 9 ♀

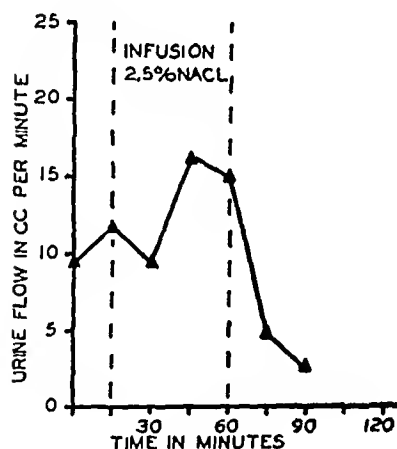


FIG. 4. Normal antidiuretic responses of 3 subjects with polydipsia and polyuria of established psychogenic origin.

patients with psychogenic and neurohypophysial polyuria and polydipsia is provided in Fig. 5.

Three subjects with well controlled diabetes mellitus and mild glycosuria were also studied (Table 1). One, without polyuria, showed the normal decrease in urine flow during the infusion. The other two had an associated polyuria and polydipsia; one exhibited a normal decrease in urine flow, the other a continued diuresis during and after the infusion.

## DISCUSSION

These experimental results, in conjunction with the experience of Hare and his associates in dogs and man, indicate that the response of hydrated

subjects to hypertonic saline infusions provides a simple, objective means of differentiating between polyuria and polydipsia of neurohypophyseal and psychogenic origin. The test is simple; it requires no special equipment, may be performed on the ambulatory patient, and is completed within three hours. Care should be taken to avoid overhydration, since the essence of the procedure consists in the release of antidiuretic substance from the

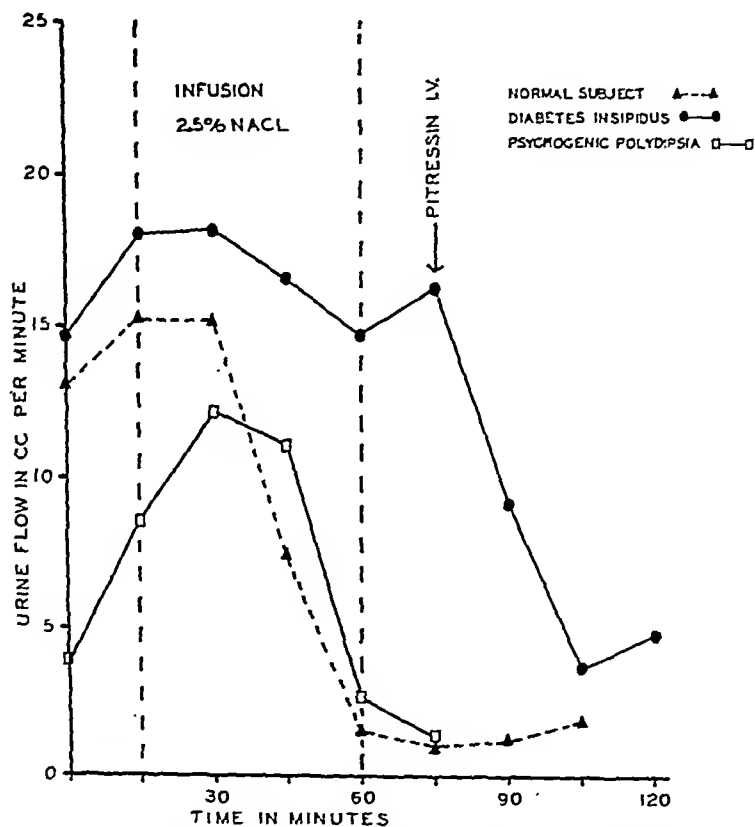


FIG. 5. Comparison of representative responses to hypertonic NaCl of the normal subject and patients with neurohypophyseal and psychogenic polyuria and polydipsia.

posterior pituitary in response to the increased osmotic pressure of the blood resulting from the hypertonic infusions. The site of action is placed by Hare (6) in the diencephalon, by others (13, 14, 15) in the osmoreceptors of the carotid.

Whether or not this procedure, in which urine flow is the only index used, will prove capable of differentiating polyuria of neurohypophyseal origin when there is an associated glycosuria or renal disease requires further investigation. In the presence of renal disease the absence of an antidiuretic



effect might be due to the inability of the tubules to respond to the posterior pituitary stimulus. Under these circumstances the tubules should also fail to respond to the administration of pitressin, thereby permitting a localization of the defect to the renal tubules. Other complications which may interfere with the interpretation of the test are those associated with

TABLE 1

Sub- ject	Age	Sex	Urine Flow in cc. per Minute per 15 Minute Period										% Change in Urine Flow†	Diagnosis
			Preinfusion		Infusion 2.5% NaCl			Post-infusion						
			I	II	III	IV	V	VI	VII	VIII	IX	X		
<i>P.D.</i>	22	M	13.0	15.2	15.1	7.3	1.5	1.0	1.2	1.8			-93.5	Normal
<i>T.C.</i>	23	M	13.2	15.6	18.5	17.2	9.9	4.7	3.8*	4.7*	4.0*		-77.0	Normal
<i>D.H.</i>	22	M	6.8	10.7	12.0	9.0	1.5	5.1	3.0				-86.0	Normal
<i>K.H.</i>	39	M	10.7	16.0	16.4	7.7	1.7	2.7	2.4				-89.5	Normal
<i>C.R.</i>	27	M	20.0	21.5	29.7	20.7	7.9	5.6	11.3				-74.0	Normal
<i>J.R.</i>	24	M	15.3	19.3	17.6	10.8	3.9	3.6	4.4	4.8*	5.8*		-76.5	Normal
<i>K.B.</i>	23	M	24.9	28.3	27.7	7.7	2.3	2.4	2.0				-93.0	Normal
<i>J.K.</i>	29	M	17.7	17.7	9.1	1.7	5.1	3.9	2.6				-90.5	Normal
<i>A.P.</i>	25	F	18.7	19.7	11.7	1.7	4.3	4.7					-91.5	Normal
<i>S.S.</i>	15	F	8.0	7.3	8.0	12.3	9.8	4.0	2.3				+18.6	Diabetes Insipidus
<i>T.K.</i>	45	M	15.3	16.6	20.0	20.0	18.6	18.6	16.6	8.0	1.0		+21.2	Diabetes Insipidus
<i>M.P.</i>	38	M	14.6	18.0	18.2	16.5	14.8	16.2	9.1	3.6	4.7		-17.8	Diabetes Insipidus
<i>M.F.</i>	13	F	15.3	15.0	15.7	16.3	15.7	14.7	11.7	10.0*	10.0	2.5*	-33.3	Diabetes Insipidus
<i>R.M.</i>	66	M	7.0	10.3	9.7	11.7	12.7	9.0	7.0	9.0*	4.5*	3.0*	-32.0	Diabetes Insipidus
<i>E.W.</i>	33	F	7.3	11.0	15.7	18.0	20.0	17.7	12.3	9.5	6.5	3.0	-13.6	Diabetes Insipidus
<i>N.B.</i>	7	M	6.3	6.3	6.7	6.6	6.7	6.2	1.6	1.0			-0.2	Diabetes Insipidus
<i>J.W.</i>	14	M	8.6	7.5	8.0	8.3	8.3	7.3	4.3*	1.2*			-15.1	Diabetes Insipidus
<i>E.K.</i>	19	F	16.0	18.0		14.0	8.3	3.0	5.3				-83.5	Psychogenic polydipsia
<i>L.G.</i>	9	F	9.46	11.7	9.34	16.34	15.0	4.73	2.53				-78.5	Psychogenic polydipsia
<i>R.H.</i>	46	F	3.8	8.5	12.1	11.0	2.6	1.3					-80.5	Psychogenic polydipsia
<i>M.B.</i>	27	F	11.0	16.0	18.0	15.7	4.3	6.0	3.5				-78.0	Diabetes mellitus
<i>R.B.</i>	13	M	11.3	13.7	17.7	4.7	5.7	6.0	5.0				-65.0	Diabetes mellitus with polyuria and polydipsia
<i>F.R.</i>	19	M	12.7	13.3	17.3	13.7	11.0	9.0	10.3	12.3	1.7	3.3*	-32.4	Diabetes mellitus with polyuria and polydipsia
<i>G.P.</i>	39	M	11.6	15.5	11.1	6.3	3.0	5.6					-80.5	Renal calculi
<i>B.K.</i>	27	F	7.3	16.6	14.3	5.13	3.93	4.9					-76.5	Renal calcinosis
<i>F.C.</i>	35	M	9.7	10.0	10.6	1.8	0.7						-93.0	Renal calculi

\* Indicates ten-minute period.

† Maximal per cent change in urine flow as compared with maximal urine flow prior to hypertonic infusion and without pitressin. Figures in bold face indicate urine flow in cc./minute after 0.1 U pitressin.

marked decreases or increases in the extracellular fluid volume, such as edema or extreme dehydration.

This procedure may prove useful in determining whether alterations in neurohypophyseal function may be involved in the antidiuretic phenomena observed in a number of conditions such as cardiac decompensation, hepatic cirrhosis and premenstrual edema. This possibility is now being explored, utilizing, in addition to urine flow, the measurement of the chloride and osmotic R/P.

## CONCLUSIONS

Evidence has been presented, derived from a study of normal subjects and patients with polyuria of both neurohypophysial and psychogenic origin, that alterations of urine flow of hydrated subjects, following hypertonic saline infusions, permit a differentiation between diabetes insipidus and psychogenic polydipsia and polyuria.

## APPENDIX

*Case Histories*

*Case 1. S.S. (N.Y.H. 419,783).* A 15-year old white school girl complained of polyuria and polydipsia of 3 years' duration. The patient had found complete relief of nocturia by taking dried posterior pituitary intranasally before retiring. Breast development, axillary and pubic hair appeared at 14 but menstruation had not begun. Physical Examination: The blood pressure was 114/72, mm. Hg. Axillary and pubic hair was scant, and the breasts were poorly developed. Laboratory Data: The blood count, urine analysis, blood chemistry, glucose tolerance test, spinal fluid analysis and visual fields were all within normal limits. Roentgen-rays of the long bones and skull were negative. The basal metabolic rate was minus 13 per cent. The concentration of 17-ketosteroids was 13.0 mg. per 24 hours. Vaginal smears showed acyclic ovarian insufficiency. The fluid intake was from 5,000 to 7,200 cc. The urine output was from 6,000 to 8,200 cc., per 24 hours, the maximal specific gravity on a prolonged concentration test being 1.006. The patient has since been well regulated by the administration of dried posterior pituitary powder by nasal insufflation three times a day.

*Case 2. T. K. (N.Y.H. 398,498).* A 45-year old white married butcher had had polyuria and polydipsia of long duration. Twelve years previously the patient first noticed nocturia, followed by polyuria and polydipsia of "several gallons a day." Shortly after the onset of symptoms, there was a marked loss of scalp hair. Polyuria and polydipsia were controlled by 10 units of pitressin subcutaneously twice a day until 2 months prior to admission, when 20 units twice a day failed to control the polyuria and polydipsia. The patient had been married 10 years and had no children. Physical Examination: The blood pressure was 150/105 mm. Hg. Generalized obesity was evident. Laboratory Data: The urine showed a faint trace of albumin, occasional RBC and from 4 to 5 WBC per high power field. The blood chemistry, glucose tolerance test and spinal fluid analysis were within normal limits. The skull roentgen-ray was negative. Maximal urine specific gravity on a standard urine concentration test was 1.005. The basal metabolic rate was plus 13 per cent. The 17-ketosteroids measured 7.6 mg. per 24 hours. Semen analysis showed a subnormal count with very poor motility. The patient has since been well regulated by the administration of dried posterior pituitary powder by nasal insufflation three times a day.

*Case 3. M.P. (N.Y.H. 334,107).* A 38-year old white, single male had suffered from polyuria and polydipsia since the age of 20. Headaches had been present since childhood as well as frequent upper respiratory infections. The patient had undergone two submucous resections and two tonsillectomies and at the age of 21, an enucleation for ophthalmitis. During the 6 years preceding his illness, he had sustained several severe blows to the head, without loss of consciousness. Physical Examination: The blood pressure was 130/80 mm. Hg. Generalized obesity was evident. He had bilateral chronic otitis

media and an artificial right eye. Laboratory Data: The urine analysis, blood count and blood chemistry were within normal limits. Roentgen-rays of skull and chest were negative. The left visual field was normal. Sinus roentgen-rays showed left ethmoidal and maxillary sinusitis. The fluid intake was from 4,000 to 13,500 cc.; urine output was from 4,050 to 10,250 cc. per 24 hours with a maximal urine concentration of 1.007. The patient has been controlled by the administration of dried posterior pituitary powder, intranasally, and pitressin tannate in oil; however, because of unpleasant reactions to all pituitary preparations, he rarely takes any antidiuretic therapy.

*Case 4. M.F. (N.Y.H. 407,007).* An 11-year old white school girl had had polyuria and polydipsia for  $3\frac{1}{2}$  months. Four months before the onset of her illness she had a fall from a bicycle, which was followed by dizziness, unilateral weakness and vomiting for several days. Spinal fluid and skull roentgen-rays at another hospital were said to be negative. The menarche occurred one month after the onset of illness. Physical Examination: The blood pressure was 104/80 mm. Hg. Moderate acne was present. The patient showed well developed secondary sexual characteristics. Laboratory Data: The blood count, blood chemistry, spinal fluid analysis and urine examination were within normal limits. Roentgen-ray of the skull was negative and the visual fields were normal. The electro-encephalogram was suggestive of defect in the frontal lobes, but equivocal. The fluid intake was from 6,300 to 12,800 cc. and the urine output from 4,475 to 11,800 cc. per 24 hours with a maximal specific gravity of 1.007. The patient was poorly regulated with pitressin, subcutaneously, and by nasal spray, but has subsequently been well controlled with dried posterior pituitary powder by nasal insufflation three times a day.

*Case 5. R.M. (N.Y.H. 404,769).* A 64-year old white, married salesman had had polyuria and polydipsia of three years duration which had been controlled with pitressin therapy. Six years previously the patient acquired syphilis and received treatment with arsenic and bismuth. The Wassermann test was negative 3 years ago. Physical Examination: Blood pressure was 160/100 mm. Hg. The fundi showed macular degeneration and hemorrhages. The heart was not enlarged and no murmurs could be detected but the peripheral vessels were sclerotic. Laboratory Data: The blood count, urine analysis, blood chemistry, skull roentgen-ray, electro-encephalogram and visual fields were within normal limits. The blood Wassermann test was equivocal and the spinal fluid Wassermann, 2+ at 0.6 dilution. Roentgen-ray of the chest showed pulmonary tuberculosis, apparently healed; gastric washings showed Gaffkey 1. The fluid intake was from 2,400 to 16,000 cc. and the urine output from 4,800 to 15,700 cc. per 24 hours with a maximal specific gravity of 1.009. The patient has since been well regulated with dried posterior pituitary powder by nasal insufflation, three times daily.

*Case 6. E.W. (N.Y.H. 513,960).* A 33-year old white, single female had suffered from polyuria and polydipsia since childhood. Breast development began at 16, sex hair appeared at 16, but the patient had never menstruated spontaneously. She had poliomyelitis at  $2\frac{1}{2}$  years, with residual weakness of left leg. She suffered from frequent headaches of considerable severity. Physical Examination: The blood pressure was 106/70 mm. Hg. The patient was a short female with generalized obesity, atrophic skin and scant body hair. Her breasts contained little glandular tissue. There was moderate atrophy of the left leg with shortening, and absent knee and ankle jerks. Laboratory Data: The urine analysis, blood count, and blood chemistry were within normal limits. Roentgen-rays of the skull were negative, but those of the long bones, revealed moderate osteoporosis. A biopsy showed atrophy of the skin. Smears showed vaginal atrophy. The fluid intake

was from 2,700 to 4,400 cc. and the urine output from 2,900 to 4,800 cc. per 24 hours with maximal specific gravity on a prolonged concentration test, 1.016. The 17-ketosteroid excretion was 10.0 mg. per 24 hours.

*Case 7. N.B. (N.F.H. 464,203).* A 7½-year old, white schoolboy had had polyuria and polydipsia since the age of 4. Bilateral otitis media had been present since the age of 9 months and had been treated with roentgen-ray, radium and two right mastoidectomies with a resultant right facial paralysis. Physical Examination: The blood pressure was 110/70 mm. Hg. There was a purulent discharge from the right ear and deafness. The right cervical lymph nodes were enlarged. Laboratory Data: The urine analysis, blood count, blood chemistry and spinal fluid analysis were within normal limits. Roentgen-rays of skull and chest were negative. Visual fields were negative. A smear of a cervical lymph node was positive for tubercle bacilli. The fluid intake was 4,660 cc. and the urine output 4,535 cc. per 24 hours, with maximal specific gravity of 1.001. The patient has since been regulated by the administration of 0.75 cc. pitressin tannate in oil twice daily.

*Case 8. J.W. (N.Y.H. 360,161).* A 14-year old, white schoolboy with polyuria and polydipsia since the age of 5 was made available for these studies through the kindness of Dr. Bronson S. Ray. At the age of 10, the patient had a right temporal hemianopsia, vision on the left being limited to light perception. At this time Dr. Ray partially resected a malignant chromophobe struma of the pituitary. This was further treated by two courses of irradiation. Polyuria and polydipsia persisted. During the previous year the patient had grown 13 cm. and gained 13 kg. Physical Examination: The blood pressure was 110/80 mm. Hg. The patient was an obese boy with fat pads over the hips and breasts, no sex hair and prepuberal genitalia. Laboratory Data: The blood count, urine analysis and blood chemistry were within normal limits. The 17-ketosteroid excretion was 3.03 mg. per 24 hours. Visual fields and roentgen-ray of the skull were within normal limits. The fluid intake was from 3,000 to 4,000 cc., and the urine output from 5,000 to 6,000 cc. per 24 hours, with a maximal specific gravity 1.005. The patient has since been regulated by the administration of dried posterior pituitary powder by nasal insufflation from 3 to 4 times daily.

*Case 9. E.K. (N.Y.H. 455,825).* A 19-year old white, female secretary had polyuria and polydipsia for from 5 to 6 years. She drank from 15 to 20 glasses of fluid a day and voided about 15 times during the day and night. The menarche occurred at the age of 13, with an excessive growth of facial hair starting at that time. Physical Examination: The blood pressure was 130/85 mm. Hg. Slight hypertrichosis was evident but the clitoris was not enlarged. Laboratory Data: The blood count and urine analysis were within normal limits. Roentgen-ray of the skull was negative. The basal metabolic rate was -9 per cent. Vaginal smears revealed an anovulatory cycle with low follicular activity. The 17-ketosteroid excretion was 10.8 mg. per 24 hours. Maximal urine specific gravity was 1.019. Since the performance of the hypertonic saline test, the fluid intake has been voluntarily reduced to 7 glasses of fluid a day without discomfort and there has been no frequency.

*Case 10. L.G. (N.Y.H. 424,561).* A 9-year old white schoolgirl had suffered from polyuria and polydipsia for 1 year and from excessive weight gain for 2 years. The fluid intake was "3 to 4 quarts a day" and frequency 14 times a day. Physical Examination: The blood pressure was 110/70 mm. Hg. The patient showed generalized obesity. Laboratory Data: The urine analysis, blood count and blood chemistry were within normal limits.

Roentgen-rays of skull and long bones were negative. The maximal urine specific gravity on a concentration test was 1,039. The patient's polyuria and polydipsia are no longer present.

*Case 11. R.H. (N.Y.H. 466,849).* A 46-year old white, registered nurse showed polyuria, polydipsia and weight gain of 3 months' duration. The fluid intake was from 3,400 to 7,500 cc., and the urine output 5,600 cc. per 24 hours. She had had menopausal symptoms for 4 years following a panhysterectomy, only partially controlled with Progynon D. H. 0.1 mg. per day. Thirty-nine years ago the patient had had "brain fever" characterized by drowsiness and fever for 9 months. Physical Examination: The blood pressure was 140/90 mm. Hg. Laboratory Data: The blood count and urine examination were essentially negative. The skull roentgen-ray was negative. Maximal urine concentration was 1.006. Since the performance of the hypertonic saline test and adequate estrogen replacement therapy, the patient has had no polyuria and polydipsia.

*Case 12. M.B. (N.Y.H. 402,056).* A 27-year old, white, married housewife with known diabetes mellitus for 3 years, was regulated with 50 units protamine zinc insulin and 50 units regular insulin daily. She had no polyuria or polydipsia. Her weight was constant. Physical Examination: Except for a diffusely enlarged thyroid there were no important findings. Laboratory Data: Urine analysis showed sugar 2+, acetone 0, and specific gravity 1.018. The blood count was within normal limits.

*Case 13. R.B. (N.Y.H. 34,863).* A 13-year old, white schoolboy entered the hospital in diabetic acidosis. After the diabetes mellitus was controlled with insulin, the patient continued to have polyuria and polydipsia. Physical Examination: Essentially negative, B.P. 120/70 mm. Hg. Laboratory Data: The urine showed sugar 1+ and specific gravity 1.014. The blood count and roentgen-ray of the skull were normal. The fluid intake was from 800 to 3,200 cc. and the urine output from 1,200 to 7,000 cc. per 24 hours. The patient has since been regulated with 45 units protamine zinc insulin, has only occasional polyuria and polydipsia, and has maintained constant weight.

*Case 14. F.R. (N.Y.H. 36,589).* A 16-year old, schoolboy had a long history of polyuria and polydipsia, loss of vision, drowsiness and frontal headaches. At the age of 13 he was obese, underdeveloped and had no secondary sexual characteristics; the fundi revealed pallor of the optic discs and bilateral macular degeneration. The patient was treated with chorionic gonadotropic hormone and methyl testosterone, with resulting development of secondary sexual characteristics. Laboratory studies and roentgen rays were negative at this time. Three years later, the patient was admitted to the hospital in diabetic acidosis. Physical Examination: The blood pressure was 114/74 mm. Hg. The fundi showed macular degeneration. The patient had the typical eunuchoid body habitus. Laboratory Data: The urine showed sugar 4+, specific gravity 1.026. The blood count was within normal limits. Roentgen-ray of the skull was negative. Examination of the visual fields revealed bilateral, bitemporal scotomata not considered due to pituitary enlargement. The fluid intake was from 3,100 to 5,100 cc. and the urine output from 2,360 to 5,050 cc. per 24 hours. The diabetes mellitus has since been controlled with 25 units protamine zinc insulin daily; however, polyuria and polydipsia have continued, but without weight loss.

*Case 15. G.P. (N.Y.H. 445,407).* A 39-year old white, male clerical worker had suffered from known renal calculi for five years. He had frequency every 2 hours, nocturia

0-3, intermittent dysuria and flank pain. Physical examination was essentially negative. The blood pressure was 120/88 mm. Hg. Laboratory Data: The urine and blood count were within normal limits. Serum calcium was 12.16 mg. per cent; phosphorus, 1.69 mg. per cent and alkaline phosphatase 3.7 Bodansky units. Urinary calcium was 350 mg./24 hours on a 130 mg. calcium diet. The maximal urine concentration was 1.021. Roentgen-ray of the skeletal system showed hypertrophic osteoarthritis of the cervical and dorsal spine and aseptic necrosis of the left scaphoid. Intravenous pyelograms revealed right renal calculi and a left renal calculus. Two normal parathyroids were removed with subsequent lowering of the serum and urinary calcium and a rise of the serum phosphorus.

*Case 16. B.K. (N.Y.H. 426,251).* A 27-year old white, married housewife had had a parathyroidectomy one year previously with removal of a parathyroid adenoma from the anterior mediastinum, for hyperparathyroidism associated with marked bone lesions and renal calcinosis. Physical examination was essentially negative. The blood pressure was 136/86 mm. Hg. Laboratory Data: The blood count, urine analysis and blood chemistry were within normal limits. The urea clearance was 53.3 per cent, and phenol sulfonphthalein test 83 per cent. The fluid intake was from 1,200 to 2,150 cc. and the urine output from 1,070 to 2,000 cc. per 24 hours with a maximal specific gravity of 1.016. Roentgen-ray of the skeleton showed healing osteitis fibrosa cystica.

*Case 17. (N.Y.H. 461,452).* A 35-year old Puerto Rican mechanic with a 10-year history of renal calculi recently had a transurethral resection with removal of vesicle calculi and a left nephrolithotomy. Physical Examination was essentially negative. The blood pressure was 120/80 mm. Hg. Laboratory Data: The urine showed a faint trace of albumin and many clumps of WBC. A count showed 100 WBC and 0-10 RBC per high power field. The blood count and blood chemistry were within normal limits. The maximal urine concentration was 1.012, and the phenolsulfonphthalein test showed a clearance of 78 per cent. A urine culture revealed Aerobacter aerogenes. Intravenous pyelograms showed fair bilateral renal function with calyceal deformity indicating old pyelonephritis.

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# THIOURACIL IN THE TREATMENT OF HYPERTHYROIDISM COMPLICATING PREGNANCY AND ITS EFFECT ON THE HUMAN FETAL THYROID

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THIOURACIL has been in use for the past 4 years. Astwood (1) has reported its use in four pregnant patients without untoward results. Palmer (15) treated 3 pregnant women, one of whom delivered a normal child. Eaton (4) has reported 2 cases in which thiouracil was used. One of these was the only case in which the child's thyroid was enlarged at delivery and remained so for several months after birth, but the child was otherwise normal. Vogt (17) treated one pregnant patient with thiouracil; although she herself developed a marked enlargement of the thyroid, the child was normal.

Only a few experimental papers have been published, including that of Goldsmith et al. (7) who recorded that newborn rats whose mothers had been treated daily with thiourea were both normal in weight and in external appearance as compared with the controls. He noted that the thyroid was slightly increased in weight and there was active hyperplasia characterized by high columnar epithelium with a limited amount of stainable colloid. These effects disappeared when the animals were placed on a laboratory stock diet.

The use of any drug during gestation always raises the question of possible toxicity of the medication for the fetus. This is especially true in regard to the thyroid. Williams (20) has demonstrated that thiouracil does pass through the placenta in rats. Hughes (9) has shown that by repeated doses of thiouracil to newborn rats it is possible to induce cretinism in these animals. This was confirmed by Freiesleben and Kjerulf-Jensen (6). The thyroid gland of these animals increased in weight and showed typical hyperplastic changes and a decrease in the colloid contents.

The time of the administration of this drug during pregnancy necessitates a knowledge of the embryonic development of the thyroid. It is not before the fifth month that follicular-like structures are present in the fetus. Colloid has been demonstrated in approximately the sixth month. The first evidences of secretion into the individual follicles are noted at

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the seventh month of intrauterine life. The gland, however, has numerous solid cell nests even at birth. Grollman (8) believes it is probable that the thyroid is functional in early embryonic life and that its dysfunction at this stage, as for example in cretins, may result in abnormality of the fetus. Means (13) states that the thyroid is probably fully functioning at birth. Zondek (23) supports this theory by citing the case of a myxedematous female whose condition markedly improved during her pregnancy. Windle (21) noted that human infants born with or without atrophic thyroids exhibit none of the symptoms of cretinism; however, a latent athyreosis soon manifests itself. It seems probable to him that, in humans, the

TABLE 1. INCIDENCE OF HYPERTHYROIDISM IN PREGNANCY

Author	Year	Region	Series of Pregnancies	Cases of Hyperthyroidism	Percentage Incidence
Markoe (12)	1918	New York	100,000	8	0.008
Yoakum (22)	1928	Michigan	937	35	3.7
Wallace (18)	1933	New York	11,571	9	0.07
Portis & Roth (16)	1939	Illinois	1,000	14	1.4
Javert (10)	1940	New York	23,439	18	0.076
McLaughlin & McGoogan (11)	1942	Nebraska	6,112	19	0.3
Whitelaw	1947	New York	13,264	8	0.06
TOTAL			156,323	111	0.071

mother's hormone is available to the fetus because it can transverse the placental barrier. The transversal of thyroglobulin is denied by others and this opposing theory is substantiated by the studies of Dorff (3) on two sets of twins in which one twin in each set was a sporadic cretin. Faxen (5) reported similar findings the following year. It is known, of course, that the onset of sporadic cretinism is insidious, and that the symptoms are usually not recognizable before the third month. Therefore, it is of the utmost importance that we know whether thiouracil or any of its derivatives could induce cretinism in the human.

Fortunately, toxic hyperthyroidism is an uncommon complication of pregnancy. The incidence of hyperthyroidism complicating pregnancy for the period 1935-45 at Binghampton City Hospital and Charles S. Wilson Memorial Hospital was 0.06 per cent. The opportunities, therefore, for evaluating any drug in the treatment of hyperthyroidism complicating pregnancy are very limited for the individual worker. The author has treated 3 pregnant hyperthyroid women with thiouracil. One aborted at

3 months, although her basal metabolism was normal; the second delivered at term a normal baby whose thyroid showed no enlargement; and the third case, because it is so unusual, is presented below:

### CASE REPORT

*G.D.* a 30-year old white female, para I, gravida II, in the 26th week of pregnancy was first admitted to the hospital on September 24, 1944 complaining of dizziness and nausea. These symptoms had first been noted approximately two years previously, together with loss of weight and mild exophthalmos, and had become markedly aggravated during the second month of this pregnancy. The patient had first noticed her goiter ten years previously, but had experienced no toxic symptoms up to two years ago. During and after her first pregnancy, five years previously, she had noticed an increase in the

TABLE 2. *Case G.D.*

	On Admission	1 Week before Delivery
Basal metabolic rate	+65 per cent	+8 per cent
Circulation time	8 sec.	14 sec.
Creatine excretion	420 mg./24 hrs.	160 mg./24 hrs.
Blood Cholesterol	105 mg./100 cc.	240 mg./100 cc.

size of her goiter. This gestation had been uneventful, however. Up to one week prior to her hospital admission the patient had not seen a physician.

Physical examination revealed a thin, markedly nervous, white female with moderate exophthalmos. Positive findings were a lid lag, a smooth and moderately enlarged thyroid, a resting pulse rate of 140 and a blood pressure 150/90 mm. Hg. The skin was moist and warm, there was a generalized increase in the reflexes and there was a fine tremor of the hands. The uterus corresponded in size to the period of amenorrhea. The fetal heart sounds were normal. Her basal metabolic rate on admission was +65 per cent. The creatine excretion was 420 mg. per 24 hours. Circulation time was 8 seconds. The patient was given general supportive treatment which included large doses of Vitamin B complex, a high-vitamin, high-caloric diet, and was given 0.6 grams daily of thiouracil<sup>1</sup> for one week, after which the dosage was reduced to 0.4 grams a day. During her stay in the hospital bi-weekly check-ups of her white blood count and differential failed to reveal any significant changes. Her condition rapidly improved and her basal metabolic rate, pulse and blood chemistry, as well as circulation time were within normal range at the time of delivery (Table 2). Parturition was uneventful. The baby was an anencephalic male monster<sup>2</sup> with a cleft palate born the 41st week of pregnancy. Its weight was 2736 grams. It lived six hours. Permission was granted for removal of the thyroid only, which was dissected out 10 minutes after death. The wet, fresh gland weighed .828 grams. It was then fixed for an analysis of its iodine content and for microscopic study (Figs. 1 & 2). The iodine values of two different sections were 5.58 and 5.59 mg. of iodine per 100 grams

<sup>1</sup> I wish to thank Dr. Hardy for the thiouracil used in this study.

<sup>2</sup> Dean (2) has reported a relationship of two cases of anencephalic monstrosities born to the same woman suffering from hyperthyroidism.

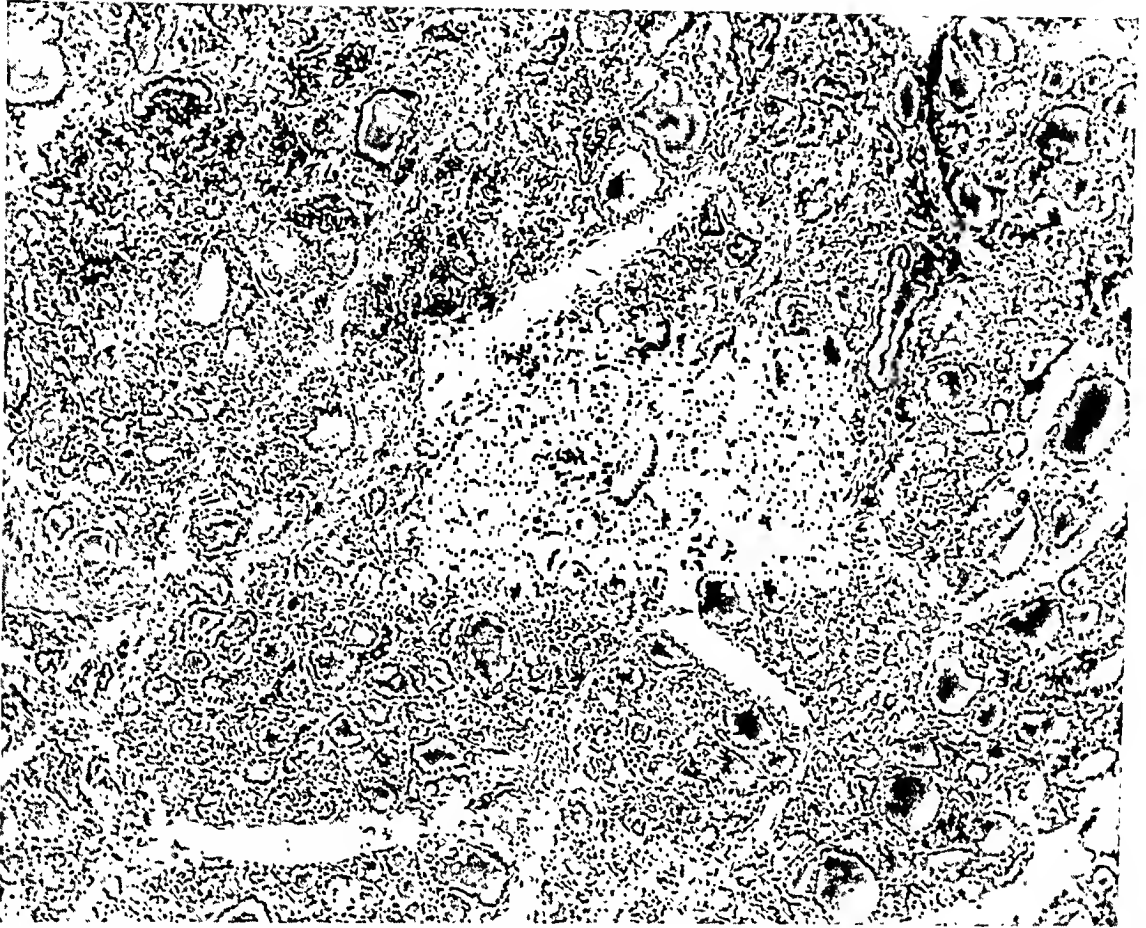


FIG. 1. Photomicrograph of a section of thyroid of a newborn from a mother with hyperthyroidism (*Case G.D.*) ( $\times 150$ )

of wet tissue. This is well within the range of iodine values for the thyroid of the normal newborn. Histological sections of the gland revealed little change from that found in the thyroid of a corresponding newborn infant.

#### DISCUSSION

In this markedly toxic hyperthyroid pregnant female, thiouracil, used over a period of three months, brought the basal metabolic rate down to normal and maintained it. This is in contrast to iodine medication, where it has been pointed out by Means and Lerman (14) that the lowest value reached is still above the normal range. Wieland (19) has reported in his study on weights of newborn thyroid that they all exceed 1 gram. Nearly all authors, both in this country and abroad, are agreed that the normal gland of the newborn weighs approximately one gram or slightly more in the wet, fresh state. Therefore, it can be seen that there was no increase in the weight of this specimen over the average normal. In fact it was some-

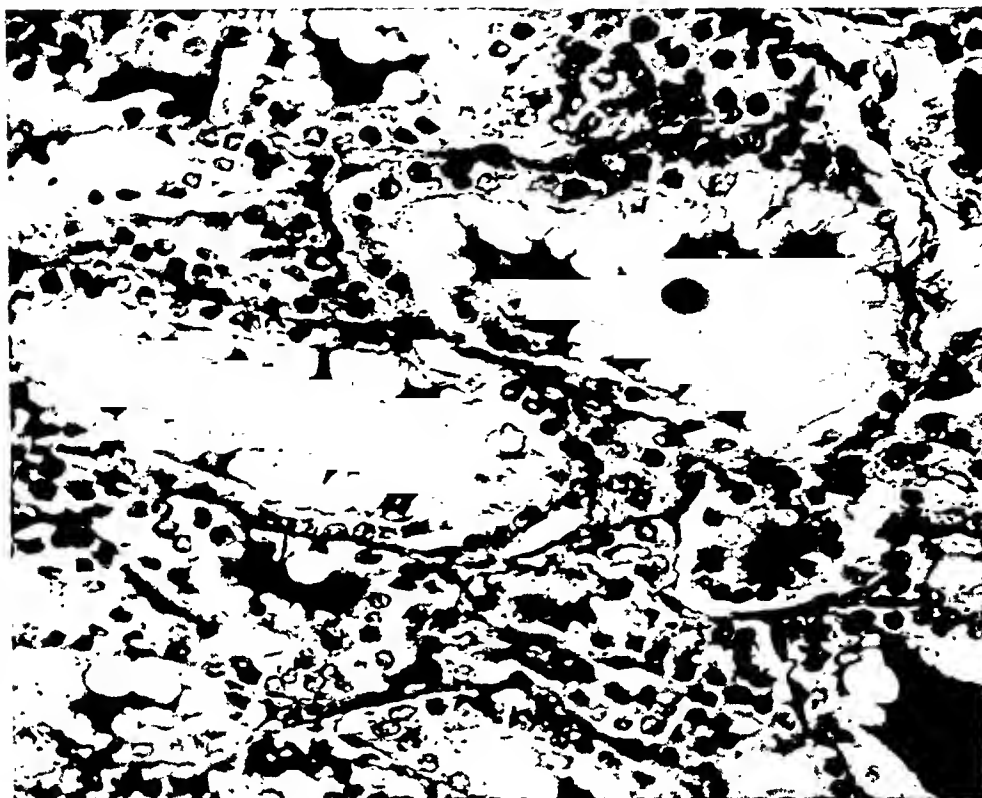
FIG. 2. Same as Figure 1. ( $\times 770$ )

TABLE 3. THYROID WEIGHTS OF NEWBORN

City or Country	Wet Weight
	<i>gm.</i>
Kiel (Wegelin)	1.9
Königsburg (Wegelin)	3.5
Berlin (Kloeppel)	5.7
Freiburg (Kloeppel)	10.7
Munich (Wegelin)	6.0
Austria (Guggisberg)	1-2
Switzerland (Wegelin)	8.2
Finland (Leidenius)	3.2
Holland	1½-3
Author's case	0.828

what below average. The total iodine content, which included both inorganic and organic iodine, also showed no variation from the normal. The histologic appearance of the tissue showed that the gland had a moderate amount of colloid and gave little indication of having been subjected to prolonged thiouracil medication (Fig. 2). It would appear on the basis of this single case that thiouracil administered to the hyperthyroid pregnant female does not exert enough injurious effect on the thyroid of the human newborn to be detected either microscopically or chemically. This agrees with the experimental findings in rats reported by Freiesleben and Kjerulf-Jensen (6).

### SUMMARY

A markedly hyperthyroid pregnant female in the 26th week of gestation was treated with thiouracil up to and through delivery. She received 0.6 grams per day for one week, following which she had 0.4 grams per day for 13 weeks. At the time of delivery she was free of toxic symptoms. An anencephalic monster was delivered whose thyroid gland fell within the normal range in weight, iodine content; and in histological appearance when it was examined.

### ACKNOWLEDGMENT

I wish to thank Dr. Astwood for the iodine determinations and Dr. Carlucci for referring this patient to me for study.

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# ANNOUNCEMENT OF THE 1948 MEETING OF THE ASSOCIA- TION FOR THE STUDY OF INTERNAL SECRETIONS

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The Thirtieth Annual Meeting of the Association for the Study of Internal Secretions will be held in the Palmer House, Chicago, Illinois, June 18 and 19, 1948.

The scientific sessions will be held in the Red Lacquer Room and registration will be on the fourth floor just outside the Red Lacquer Room. The Annual Dinner will be held in the same room on Friday, June 18th at 7 p. m. and will be preceded by a cocktail party, the location of which will be announced later. The Council will meet at 2 p. m. Thursday, June 17th.

All members of the Association who plan to attend the Thirtieth Meeting are urged to make their reservations at once with the Palmer House, stating the time of arrival and how long they plan to remain in Chicago.



# Announcement of Awards and Fellowship of the Association

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## *Nominations for Awards*

Three awards for meritorious work in endocrinology will be given at the next annual meeting of the Association. A special committee of five members of the Association chooses the recipients of these Awards, subject to ratification by the Council, and each member of the Association has the privilege of making one nomination for each award.

Nominations for the Awards should be made on special application forms which may be obtained from the Secretary, Dr. Henry H. Turner, 1200 North Walker Street, Oklahoma City 3, Oklahoma. All nominations, accompanied by a statement of the importance of the nominee's contributions to endocrinology and a bibliography of his most important papers with reprints if possible, should be sent to Dr. Turner's office not later than March 15, 1948.

## THE E. R. SQUIBB AND SONS AWARD

The E. R. Squibb and Sons Award of \$1,000.00 was established in 1939. It was given in 1940 to Dr. George W. Corner; in 1941 to Dr. Philip E. Smith; in 1942 to Dr. Fred C. Koch; in 1944 to Dr. Edward A. Doisy; in 1945 to Dr. E. C. Kendall; in 1946 to Dr. Carl G. Hartman; in 1947 to Drs. Carl F. and Gerty T. Cori. No award was made in 1943. No age or special limitation is stipulated by the donor of the award.

## THE CIBA AWARD

The Ciba Award, established in 1942, is given in recognition of the meritorious accomplishment of an investigator, not over 35 years of age, in the field of clinical or pre-clinical endocrinology. In 1944 the Award was given to Dr. E. B. Astwood; in 1945 to Dr. Jane Anne Russell; in 1946 to Dr. Martin M. Hoffman and in 1947 to Dr. Choh Hao Li. The Award is for \$1,200.00. If within two years of the date of the Award, the recipient chooses to use it to aid in working in a laboratory other than the one in which he normally is located, the Award will be increased to \$1,800.00.

## THE AYERST, McKENNA & HARRISON FELLOWSHIP

The first award of the Ayerst, McKenna & Harrison Fellowship was given to Dr. Samuel Dvoskin in 1947. The fellowship was founded in order to encourage investigation in the field of endocrinology rather than as an award



for work done. The amount of the fellowship is \$2,500.00 annually. The nominee must possess the degree of Doctor of Philosophy or Doctor of Medicine or their equivalent. It is suggested that no restriction be placed on age, but that preference be given to applicants who have recently completed the requirements for their Ph. D. or M. D. degree. The nominee must present evidence of scientific ability as attested by studies completed or in progress and/or the recommendation of responsible individuals; submit a program of proposed study; indicate one or more institutions where the proposed program will be carried out; submit statement of approval from the investigators with whom he proposes to conduct his research; serve full time if awarded a fellowship. A small amount of time (10 to 15 per cent) may be allotted for course work or for participation in teaching, the latter purely on a voluntary basis.



# Postgraduate Course in Endocrinology

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The Postgraduate Committee of THE ASSOCIATION FOR THE STUDY OF INTERNAL SECRETIONS, under authority of its Council, announces a course of lectures and demonstrations in CLINICAL ENDOCRINOLOGY to be held in LOS ANGELES at the BILTMORE HOTEL, FEBRUARY 23, to 28, 1948, inclusive.

The faculty will consist of the most prominent investigators and clinical endocrinologists in the various branches of the medical sciences in the United States and Canada.

It is the intent of the Committee that this course be a practical one of interest and value to both the GENERAL PRACTITIONER AND THE SPECIALIST.

A fee of \$100 will be charged for the entire course and the attendance will be limited to 100. Registration will be in the order of checks received and will close on February 1, 1948. Should there be an insufficient number of applicants to warrant the course, the registration fee will be immediately refunded in full.

Please make your application on your letterhead and forward, together with your check payable to THE ASSOCIATION FOR THE STUDY OF INTERNAL SECRETIONS, to DR. E. KOST SHELTON, CHAIRMAN of the POSTGRADUATE COMMITTEE, 921 WESTWOOD BOULEVARD, LOS ANGELES 24, CALIFORNIA.

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Since satisfactory hotel accommodations are still difficult to procure on short notice in Los Angeles, especially during the winter season, it is suggested that all applicants MAKE THEIR RESERVATIONS EARLY.

SOME LARGE HOTELS IN THE METROPOLITAN AREA OF  
LOS ANGELES:

Alexandria  
Ambassador  
Biltmore

Chapman Park  
Gaylord  
Hayward

Lankershim  
Mayflower  
Town House

AMERICAN ASSOCIATION FOR THE STUDY OF GOITER  
VAN METER PRIZE AWARD

The American Association for the Study of Goiter again offers the Van Meter Prize Award of Three Hundred Dollars and two honorable mentions for the best essays submitted concerning original work on problems related to the thyroid gland. The Award will be made at the annual meeting of the Association which will be held in Toronto, Canada, May 6th, 7th, 8th, 1948 providing essays of sufficient merit are presented in competition.

The competing essays may cover either clinical or research investigations; should not exceed three thousand words in length; must be presented in English; and a typewritten double spaced copy sent to the corresponding secretary, Dr. T. C. Davison, 207 Doctors Building, Atlanta 3, Georgia not later than February 1st, 1948. The committee, who will review the manuscripts, is composed of men well qualified to judge the merits of the competing essays.

A place will be reserved on the program of the annual meeting for presentation of the Prize Award Essay by the author if it is possible for him to attend. The essay will be published in the annual Proceedings of the Association. This will not prevent its further publication, however, in any Journal selected by the author.

T. C. DAVISON,  
*Corresponding Secretary*



## Abstracts of

## CURRENT ENDOCRINE LITERATURE

*Editor*; D. A. MCGINTY. *Collaborators*: A. R. ABARBANEL, F. N. ANDREWS, B. L. BAKER, F. A. DE LA BALZE, ISRAEL BRAM, R. A. CLEGHORN, RUCKER CLEVELAND, C. D. DAVIS, ANNA FORBES, M. B. GORDON, H. S. GUTERMAN, M. M. HOFFMAN, R. G. HOSKINS, C. D. KOCHAKIAN, H. S. KUPPERMAN, H. L. MASON, JANET W. MCARTHUR, THOMAS H. MCGAVACK, A. E. MEYER, K. E. PASCHKIS, A. B. PINTO, J. R. REFORZOMEMBRIVES, E. C. REIFENSTEIN, JR., G. G. RUDOLPH, L. T. SAMUELS.

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### PANCREAS

STAINSBY, W. J. AND J. A. COLLINS, JR. Diabetes mellitus. An analysis of 250 cases and a discussion of simplified procedures for regulation. *Pennsylvania M. J.* 50 (8): 808-811 (1947).

An analysis of 250 consecutive cases of diabetes admitted to the Geisinger Memorial Hospital showed that the main reasons such patients were unsatisfactorily regulated were: (1) insufficient diabetic instruction; (2) too complicated a dietary and insulin regime. Experience has shown that a simple diet is satisfactory for the regulation of most cases and it may be quite similar to that taken prior to the development of diabetes. The main point is to take approximately the same amount of calories daily with the same proportions of carbohydrate, protein, and fat. The same simple procedures apply to the use of insulin, using single injections of protamine zinc insulin whenever possible and simple mixtures of protamine zinc insulin and crystalline insulin for most of the others. For severe cases of diabetes two injections of insulin each morning, one of protamine zinc insulin, the other of an intermediate insulin, such as globin insulin, have been found most practical. Mild hyperglycemia in the absence of ketosis and unfavorable symptoms is in no way harmful to patients. Insulin reactions in the elderly are dangerous and should be avoided as much as possible.—(Authors' Conclusions)—I.B.

WOOD, M. N. Chronic peptic ulcer in 94 diabetics. *Am. J. Digest Dis. and Nutrition.* 14 (1): 1-11 (1947).

The author studied 94 diabetic patients with chronic peptic ulcer. He noted that the average age of these patients was greater than the age of the average ulcer group, and there was a relatively high incidence of females with ulcers in his study. The initial ulcer symptoms were vague and atypical, especially in those patients with diabetes of long standing. 21 of the patients had no free hydrochloric acid in the fasting gastric contents. The high incidence of ulcer complications (hemorrhage, perforation, and obstruction) was suggested as being due to the vagueness of symptoms which masked the diagnosis of the ulcer and the duodenal obstruction. Arteriosclerosis was considered a contributing factor to bleeding from the peptic ulcers studied. Therapy of these patients was directed primarily toward relieving the ulcer symptoms, with insulin added to control the hyperglycemia and glycosuria.—H.S.G.

## GENERAL

HANSON-PRUSS, O. C. Thiouracil in the treatment of leukemia. *Proc. Soc. Exp. Biol. and Med.* 64 (4): 496-500 (1947).

Six patients with chronic myelogenous leukemia and one with acute myeloblastic leukemia were treated with thiouracil. The average daily dose was 1.5 Gm., and one patient received a total of 359.3 Gm. over a 10 month period. Thiouracil was well tolerated by every patient and had no effect on the total circulating white cell count, nor on the differential white cell picture, red cell elements, hemoglobin or platelet values or the bone marrow pattern. At the dosage given, the drug had little influence on the B.M.R., serum cholesterol, bone marrow pattern or myeloid dysfunction and did not modify the clinical course of the disease. The author concluded that thiouracil has no value as an adjunct in the treatment of myelogenous leukemia.—*F.N.A.*

LIKINS, C. H., JR., E. P. SCOTT, AND N. I. HANDELMAN. Laurence-Moon-Biedl syndrome. *Am. J. Dis. Child.* 73: 195 (1947).

The authors present the case histories and physical findings of a 10½-year-old white girl with Laurence-Moon-Biedl syndrome, and in addition that of her 18-month-old brother who also had this condition. The authors briefly review the literature on this disorder. The characteristic symptoms are obesity, retinal degeneration, general hypoplasia, polydactylism and mental retardation. Frequently associated are nystagmus, strabismus, deafness and syndactylism. A familial tendency has been reported in 80 per cent of the cases. The diagnosis depends mainly on clinical observation of the abnormalities. The most acceptable concept as to etiology is that the syndrome is a hereditary developmental disturbance due to recessive mutations of two genes in the same chromosome. Among the other causal factors which have been suggested are endocrine imbalance, and cerebral aplasia from hydrocephalus. The only consistent laboratory finding has been a low basal metabolic rate. Thyroid medication has been of some value in reducing the weight of these patients, but no other endocrine therapy has been particularly helpful. The patients described by the authors received no treatment.—*E.C.R., Jr.*

WETZEL, N. C. The baby grid. An application of the grid technique to growth and development in infants. *J. Pediat.* 29: 439 (1946).

The author has adapted to individual infants a chart for direct reading of the quality of growth and development during the first three years. This chart follows the principles previously described by him and referred to as the Grid Technique (*J.A.M.A.* 116: 1187 (1941); *J. Pediat.* 22: 82, 208, 329 (1943)). The chart contains a channel on which the measurements of the baby's weight and length can be placed, and from which the developmental level, the motor-mental development, and the caloric intake can be read. Tables are also provided on which the chest circumference and the head circumference can be noted. An example of the application of the Baby Grid to the development of an infant is given. The author states that the Baby Grid has been designed to pictorialize the growth and development of a given infant during its first three years, and to make this and the quality of growth evident at a single glance.—*E.C.R., Jr.*

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## SPERMATOGENESIS IN A "PAN-HYPOPITUITARY" EUNUCHOID, AS THE RESULT OF TESTOSTERONE THERAPY

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**F**OLLOWING the report of Walsh, Cuyler and McCullagh (2) that androgen administration resulted in stimulation of the germinal epithelium of the seminiferous tubules, numerous papers have appeared verifying and amplifying this observation. Recently Simpson and Evans (1) reported active spermatogenesis in rats hypophysectomized at 40 days of age, and then injected with testosterone propionate. To the best of our knowledge, there are no reports showing a similar train of events in humans.

We wish, at this time, to report testosterone-induced spermatogenesis in a 25 year old male showing both laboratory and clinical evidence of "pan-hypopituitarism," using the term in a very broad sense.

### CLINICAL AND LABORATORY DATA

Patient, E. N., age 24, was first seen (through the courtesy of Dr. Herbert Evans) in August, 1946, at which time his major complaint was that of relative dwarfism and complete sexual infantilism.

His past medical history was essentially negative.

He weighed 8½ pounds at birth and grew normally until the age of 9 years. Since that time his growth had lagged far behind that of his contemporaries, although he believed that he had never completely ceased to grow.

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He had not noticed any axillary or pubic hair, enlargement of the penis or testes, or deepening of the voice. He had felt generally well and had noticed no unusual susceptibility to infection. Upper respiratory infections had not given rise to symptoms which one would expect in the case of adrenal cortical insufficiency.

Physical examination showed an immature male who could readily pass for a prepuberal boy of 12 or 13 years except for the presence of a "café-au-lait" coloration and rather deep lining and dryness of the skin such as one finds in the typical "pan-hypopituitary" individual. He weighed 96½ pounds with a height of 62½ inches and a span of

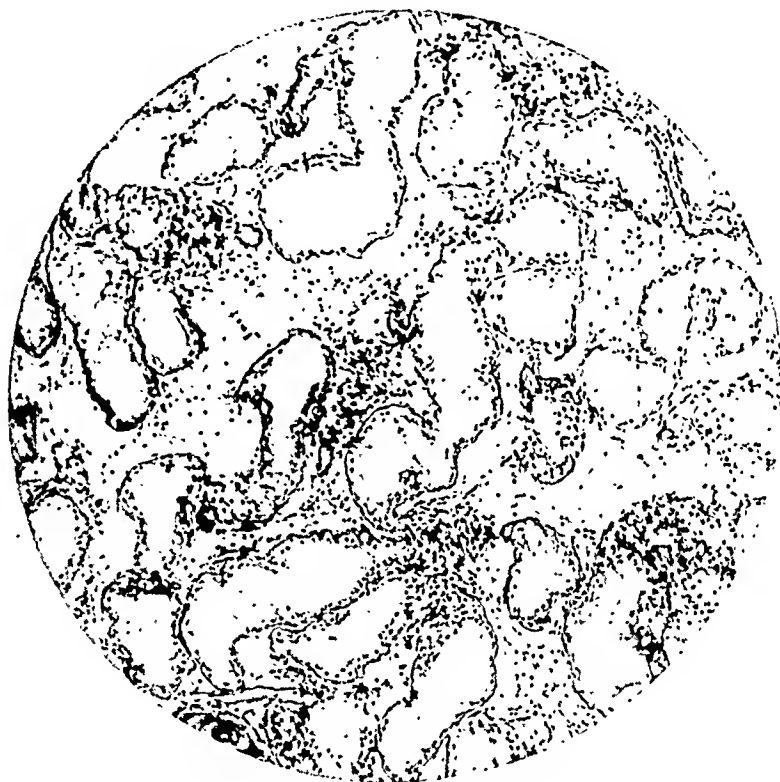


FIG. 1. Testicular biopsy before therapy ( $\times 100$ )

65 inches. There was slight breast enlargement which he believed had been present for some years. His voice was high; he possessed no facial, axillary or pubic hair. The external genitalia were infantile and no prostate could be palpated. His general muscular development was quite poor.

The patient had had no previous therapy except for a series of injections five years previously which he thought might have resulted in a slight increase in growth. He had no data, however, which would support this view.

Roentgen rays of the skeleton showed many open epiphyses with a bone age of 14 years or less. His 17-ketosteroid excretion was approximately 1 mg. per 24 hours and urinary gonadotropin excretion less than 3 mouse units per 24 hours.

A testicular biopsy done on September 10, 1946, showed a picture interpreted as normal prepuberal testes (Fig. 1).

It was decided to put the patient on a rather prolonged period of pregnant-mare-serum gonadotropin with the thought of getting some initial spermatogenic tubular development and then at a later date to maintain the patient on an adequate dosage of

testosterone. He accordingly was started on 20 units of "Gonadogen"<sup>1</sup> daily. This dose was continued for 30 days. Treatment was stopped for one week, followed by 50 units of "Gonadogen" daily for 4 weeks. It will be noted from the chart that during this medication the patient gained a small amount of weight and height (Fig. 2). There was, however, no other objective evidence of any effect of the medication. A testicular biopsy was obtained on the last day of gonadotropin therapy (Fig. 3). No significant change was noted as compared to the pretreatment biopsy. The urinary gonadotropin assay done at the end of gonadotropin therapy was negative for 4 mouse units per 24 hours and the ketosteroid excretion at this time was less than 0.5 mg. per 24 hours.

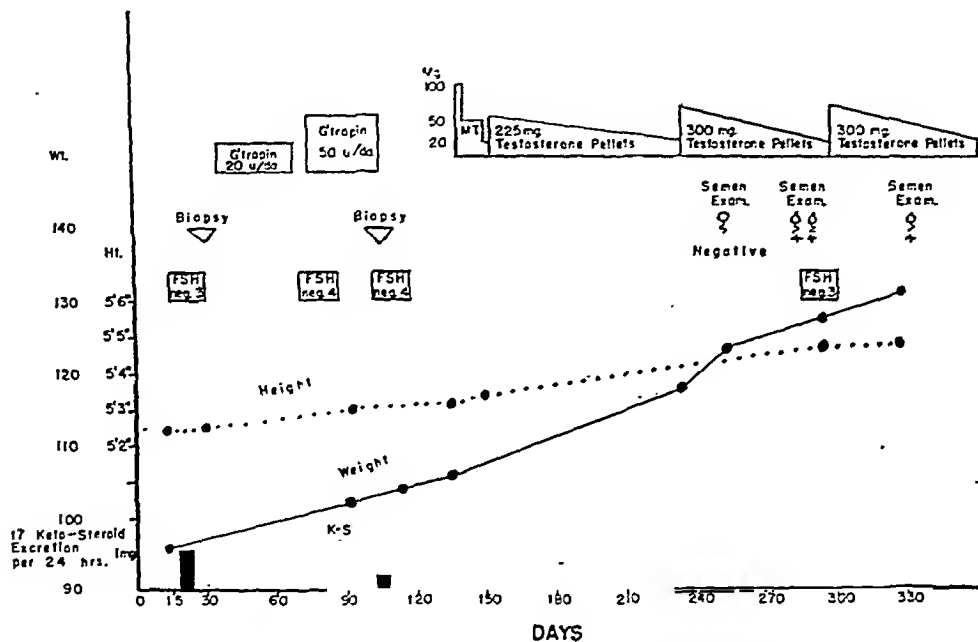


FIG. 2. Progress of patient E. N. over an eleven month period.

All therapy was discontinued for a period of one month, during which time he continued to gain weight at the same rate as had been the case during gonadotropin therapy. On December 17, 1946, he was placed on methyl testosterone<sup>2</sup> by mouth for a period of 18 days followed by testosterone pellet implantation on January 4, 1947, March 24, 1947, and on May 24, 1947 (Fig. 2).

It will be noted from the chart and from photographs after four months of testosterone therapy that he experienced a significant gain both in height and weight during his testosterone therapy (Fig. 4). The penis during this period enlarged several fold, the testes became somewhat larger and after four months of therapy his prostate was within the lower limits of normal in size.

<sup>1</sup> This preparation was supplied through the courtesy of the Upjohn Company. The "Units" are Cartland-Nelson Units, 1 Unit being equivalent to approximately 20 International Units.

<sup>2</sup> The preparations of testosterone used in this study were supplied through the courtesy of Dr. Henderson of the Sehering Corporation.



He had become aware of an occasional nocturnal emission after about 3 months on testosterone. On April 12, 1947, a specimen of ejaculate was obtained and examined grossly. No mature sperm were found in the unconcentrated specimen. On May 10, 1947, an ejaculate was again examined and a count of approximately 2,000,000 mature sperm per cubic cm. was noted. The total volume of ejaculate was less than 0.5 cc. An ejaculate was again examined on May 20. At this time the count had increased to 15,000,000 per cubic cm. and the total volume to almost 1 cc. A gonadotropin assay performed on a 24 hour specimen of urine collected on May 25 was negative for 3 mouse units of gonadotropin per 24 hours.

Reexamination of ejaculate on June 27 showed 18,000,000 mature sperm per cubic

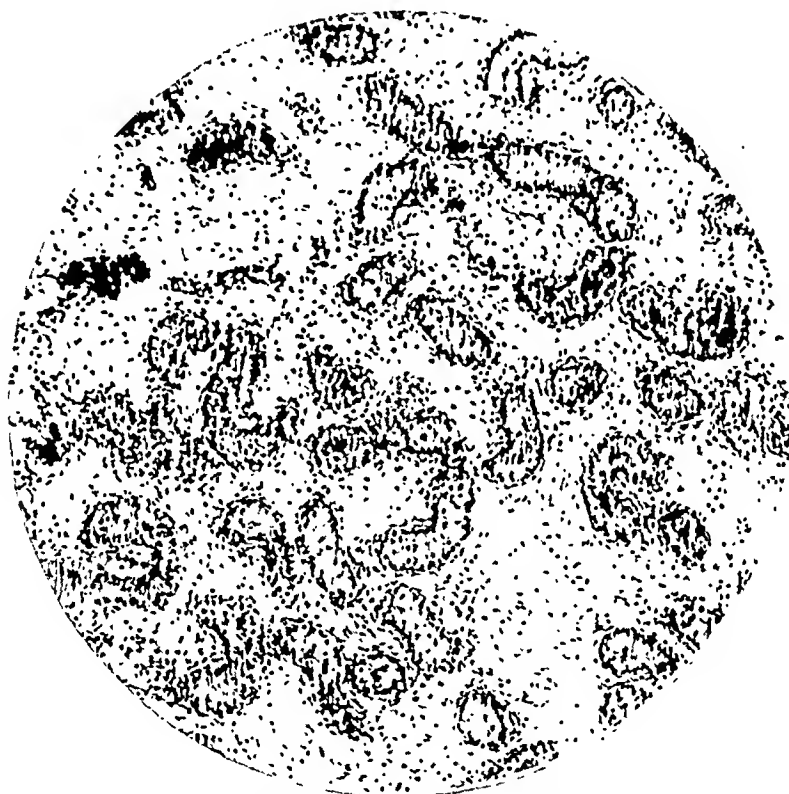


FIG. 3. Testicular biopsy after treatment for two months with pregnant mare serum ( $\times 140$ )

cm. The total volume of ejaculate was 1.2 cc. A final testicular biopsy will be performed when spermatogenesis has apparently reached a maximum level, as indicated by serial sperm counts.

### DISCUSSION

With the exception of the demonstration of spermatogenesis, all the results obtained in this patient have been reported by many observers on individuals with varying degrees of "pan-hypopituitarism" again using this term in a very loose and broad sense. With the demonstration of normal sperm in this individual, the immediate question was obviously whether testosterone alone had been responsible, through a direct effect

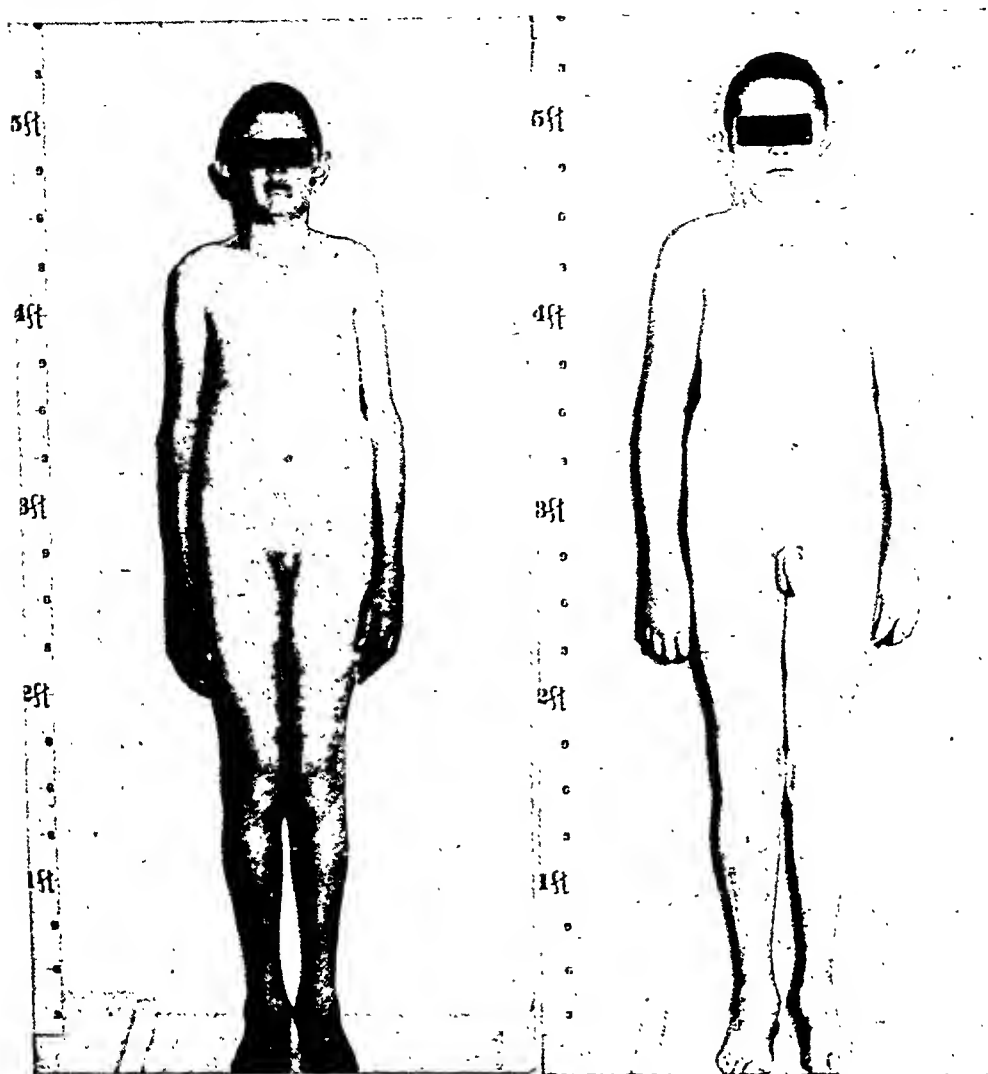


FIG. 4. Patient E. N., age 24. A. Before treatment. B. After four months of testosterone therapy.

upon the seminiferous tubules or whether his own pituitary had been stimulated by testosterone to produce gonadotropin. The negative gonadotropin titer in the urine taken after the individual had been on testosterone for five months and at a time when he had a sperm count of 15,000,000 per cc. would seem to indicate beyond any reasonable doubt that testosterone produced spermatogenesis by direct effect upon the seminiferous tubules.

The slight increase in height and weight noted during the period of "Gonadogen" administration may indicate some slight growth response to this preparation. In view of the lack of significant change in the testicu-

lar biopsy and the lack of increase in 17-kestosteroid excretion after 2 months on this preparation, there are no grounds for believing that such growth was produced by way of Leydig cell stimulation.

On the basis of the findings herein reported, there would seem to be no reason to believe that testosterone in physiologic dosage is in any way injurious to the seminiferous tubules. This observation is perhaps of some significance in view of the confused state of the literature. It would suggest that the careful clinical use of testosterone in the treatment of male sterility attributable to inadequate androgen production may be a sound procedure. As yet unpublished data from this clinic substantiate this inference.

### SUMMARY

Testosterone in the form of plain testosterone pellets (Schering) administered to a "pan-hypopituitary" eunuchoid over a period of five months produced significant spermatogenesis. The urinary gonadotropin excretion prior to and during therapy was consistently negative for 4 or less mouse units per 24 hours. It is therefore assumed that testosterone can produce spermatogenesis by direct effect upon the human seminiferous tubules without mediation by gonadotropin.

### ACKNOWLEDGMENT

We wish to express our appreciation to Dr. John Shaver for his preparation of the histological sections, and to Dr. Miriam Simpson for her aid in evaluation of biopsy material.

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# THE ENDOCRINE DISORDERS ASSOCIATED WITH CUSHING'S SYNDROME AND VIRILISM

## REPORT OF AN UNUSUAL CASE

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THE nature of the pathological disturbances which are responsible for Cushing's syndrome and virilism is controversial. It therefore seems justifiable to report an unusual case in the hope that it may throw further light on this difficult question.

### Case Report

An unmarried woman, age 38, was admitted to the London Hospital under the Medical Unit (Professor Arthur Ellis) on March 11, 1938. She had had a previous illness in the autumn of 1932. This commenced with amenorrhea and in the course of about three months she became grossly obese. Her mother subsequently volunteered that it was remarkable that her limbs had remained thin. Her face became dark red and she grew a moustache and beard. Broad lines appeared on her abdomen which she remembered particularly on account of their purple coloration, and she developed many bruises on her arms and legs without any apparent cause. She also had crops of "pimples" on her face, back and chest. During this illness she visited her panel doctor, Dr. Perchman, who subsequently wrote: "She was then very obese looking with marked double chin, hypertrichosis, dusky skin, but not very fat in the limbs. B.P. 140/100, no sugar in urine. She seemed to me to be a case of Cushing's pituitary-adrenal syndrome."

She refused to visit a hospital and the symptoms lasted for from two to two and a half years. Her appearance is shown in Fig. 1. Then the obesity, red color, striae and spots all disappeared and menstruation again became normal. Most of the abnormal hair on the face also disappeared, but she was left with a slight moustache. She remained well until November, 1937, when she again failed to menstruate. She had a scanty menstrual period in December and again in January, 1938, but subsequently she had complete amenorrhea. In January, 1938, she developed a sudden cough and pain in the right shoulder. Her doctor reported a tachycardia of 144 without fever. Subsequently she had all her teeth removed and a few days later she collapsed with precordial pain, dyspnea and multiple joint-pains. Her doctor found her cyanosed and she had a temperature of 103° F and a pulse rate of 140 per minute. About this time the hair began to increase on her face, but it never became as marked as in her previous illness in 1932. She remained in bed becoming progressively worse but her weight was stationary around 143 lbs. Her bowels were regular and her appetite remained good until just before admission when she developed edema of the legs and became disoriented.

On examination she was orthopnoeic with slight cyanosis of the lips and a malar flush but no abnormal pigmentation of the skin. There was abundant stiff black hair on the sides of the cheeks, upper lip, chin and axillae and the pubic hair extended up to the

umbilicus. A little acne vulgaris was present on the cheeks, forehead and chest. There was moderate obesity of the face, neck, trunk and limbs and a small pad of fat over the lower cervical vertebrae. A few faint bluish white striae were present in the skin of the abdomen. There was gross pitting edema of the legs extending up to the lower part of the abdomen and lumbar region. The pulse was regular at 120 to 140 per minute, the heart sounds normal and blood pressure 130/80 mm. Hg. There were signs of fluid at the base of the right lung. The veins in the right lower abdominal wall were dilated but



FIG. 1.

there were no definite signs of ascites. The liver edge was palpable from 3 to 4 inches below the right costal margin and was tender. There was some resistance in the right lower quadrant but no definite palpable mass. Apart from the mental disorientation the central nervous system was normal. The hemoglobin was from 61 to 76 per cent with from 3,000,000 to 3,800,000 red blood cells per cu. mm. The white count was normal. The plasma proteins were 5.75 gm. per 100 cc. The pleural effusion contained 90 per cent lymphocytes and was sterile. She was incontinent and an estimation of 17-ketosteroid output was not made. Her mental and general condition deteriorated rapidly and she died 17 days after admission.

## SUMMARY OF NECROPSY. (P.M. 140. 1938)

By Professor Dorothy Russell

*Edema and collapse of the lungs. Carcinoma of suprarenal cortex.*

A mass (12 × 12 × 8.5 cm.) of lobulated growth, with a thin fibrous capsule, and composed of soft opaque yellow necrotic or white and pinkish-grey tissue containing haemorrhages and a few cysts, replaced the right suprarenal body. The growth had invaded the inferior vena cava downwards for 4 cm. below the right suprarenal vein, and upwards for 10 cm. to fill the greatly dilated intra-hepatic part of the vena cava and just projected into the right auricle. There was conspicuous edema of the lower limbs and of the lumbar region. A few nodules of secondary growth were present in both lobes of the left lung and, more numerous, in the right lung. An embolus of growth was lightly adherent to bifurcation of the right pulmonary artery in the hilum of the lung. There was complete collapse of the lower lobe of the right lung and partial collapse of the lower lobe of the left lung. There was a slightly blood-stained right pleural effusion (8 oz.) and ascites (28 oz.). There were nodules of secondary growth in the liver. There was back pressure congestion and, microscopically, slight focal fatty change in the liver. There was no hypertrophy of the heart and slight general atheroma. Congestion and postmortem degeneration were present in the kidneys with, microscopically, slight focal fatty degeneration of the epithelium in the loops of Henle. There was congestion and, microscopically, slight infiltration of the pulp of the spleen with plasma cells and eosinophil leucocytes. The thymus was mainly adipose but showed, microscopically, a few cords of glandular tissue. There was abundant colloid in the normal thyroid and the four parathyroids were normal. There was great cortical atrophy in the left suprarenal body showing, microscopically, great irregularity of zonal architecture and considerable increase of fibrous stroma, and a patchy distribution of doubly refractive sudanophil lipid and very scanty pigment. The pituitary was of normal size and macroscopic appearance and the pancreas was normal. The uterus was normal and a few follicular cysts and corpora atretica were present in the ovaries. There was scanty glandular tissue in the breasts. The brain was normal. Red marrow was present in the upper third of the shaft and in the neck of the right femur. Healed fractures were present in five ribs on the left, and six ribs on the right with osteophytic outgrowths projecting into the intercostal spaces at three sites. No recognizable osteoporosis was present in the ribs or vertebrae. The middle ears and accessory air-sinuses of the skull were normal. There was hirsuties of the face in the beard and moustache area. There was abundant hair in the axillae and, of masculine distribution, in the pubic region and less marked hirsuties of the thighs and forearms. The labia majora were prominent and the clitoris slightly enlarged, the glans measuring 1.4 cm. in length, and the corpus cavernosum 3 cm. back to the pubic arch. There was slight obesity of the trunk and lower limbs. There were narrow striae on the lower abdomen and upper part of the thighs.

*Weights:* Left kidney, 204.7 Gm.; left suprarenal, 2.05 Gm.; spleen 224.2 Gm.; pituitary, 0.7 Gm.; thyroid 25.2 Gm.; thymus 43.05 Gm.; pancreas, 79.6 Gm.; ovaries 8 Gm.; brain 1452 Gm.

## MICROSCOPIC EXAMINATION

*Right suprarenal tumor.* Much of the tissue was necrotic. The surviving tumor cells collected about the blood-vessels, which were engorged, were polymorphic including spindle-forms, and mostly measured from 8 to 12  $\mu$  in diameter. Their boundaries were

ill-defined, the cytoplasm being flocculent, eosinophilic and occasionally vacuolated. Sudanophilic, isotrophic droplets were abundant in frozen sections in areas where degeneration was in progress. Unstained anisotropic lipoid was also abundant and occupied many of the better preserved cells. In sections stained for glycogen by Best's carmine method a moderate amount was present in the cytoplasm in a few restricted foci. The nuclei of the cells were relatively large and contained a heavy net of chromatin and inconspicuous nucleoli. Large uninucleate and multinucleate giant-cells were rather numerous, and in these the nuclei often contained conspicuously large eosinophilic nucleoli. The connective tissue stroma of the tumor was scanty.

A secondary deposit in the liver showed similar histological appearances.

The endocrine glands were all examined microscopically and, with the exception of the pituitary, the results of the examination have already been noted above.

The anterior lobe of the pituitary, stained with acid-fuchsin aniline-blue, was well preserved. Under low powers of the microscope, the basophilic cells appeared to be numerically reduced. A differential count was not made. With high magnifications the majority of the basophilic cells appeared to be normal mature examples. But a good many transitional basophils with fine, sky-blue granules were also present. A small minority of the cells showed the hyaline cytoplasmic change characteristic of Cushing's syndrome (4); in most instances the change was but slightly developed though in a few it was conspicuous. The other cellular constituents of the pituitary appeared normal.

## DISCUSSION

The development of Cushing's syndrome and of virilism at different times in the same individual is interesting. At the height of basophilism our patient had characteristic obesity sparing the limbs, a florid dusky complexion, facial hirsuties, acne vulgaris, a tendency to bruise easily, amenorrhea, and presumably osteoporosis. The symptoms lasted for about two and a half years, and then disappeared spontaneously for two and a half years, after which the facial hirsuties and amenorrhea recurred, and she died in about four months with a metastasizing carcinoma of the right adrenal cortex.

It might be argued that the symptoms of Cushing's syndrome had recurred but were masked by the malignant growth resulting in anemia and the absence of obesity. When Cushing's syndrome occurs with a malignant adrenal cortical tumor the patient may lose weight rapidly, but in two such cases seen by the author the patients volunteered the information that their faces were fatter and they presented other unmistakable signs of Cushing's syndrome. Conversely it might be argued that the amenorrhea which was late in developing was the result of the malignant growth and the only endocrine symptom present was the hirsuties which she had had since she first developed Cushing's syndrome. Her mother confirmed that the moustache and beard practically disappeared after the Cushing's syndrome was cured and it increased with the symptoms of adrenal tumor and was marked at the time of her death. It therefore seems justifiable to claim that she had virilism at the time of her death.

The disorder of function which led to this train of events is not clear but it is well known that both Cushing's syndrome and virilism may be associated with either hyperplasia or neoplasm of the adrenal cortex. Moreover it is fair to assume that when the patient had Cushing's syndrome the basophilic cells in the anterior lobe of her pituitary gland had undergone the characteristic hyaline change described by the author (4) but, at autopsy only minimal changes were found associated with virilism. This conforms with our previous experience, and with that of Thompson and Eisenhardt (16) who examined the pituitary glands in a large series of cases of Cushing's syndrome and virilism. It may be assumed, therefore, that this change represents an alteration in the physiological activity of the basophilic cells in Cushing's syndrome which is not present in virilism. We considered that the hyaline basophilic cells appear to be healthy and not degenerating, a view shared by others on cytological grounds (12, 13, 14). Moreover similar cells occurred in the minute fragments of pituitary which remained after incomplete hypophysectomy in rats (8). These rats were still growing, albeit at a reduced rate, and their pituitary fragments must have been working hard to permit any growth to occur. We therefore concluded that hyaline cells are associated with an increased function of the pituitary gland.

Both Albright (1) and Kepler (11) have stated on theoretical grounds that the hyaline basophilic cells represent a secondary degenerative condition and that the primary disorder is in the adrenal cortex. Heinbecker (10), who also believed that the pituitary change is degenerative, claimed that it is due to a primary lesion in the hypothalamus of patients dying of Cushing's syndrome without adrenal cortical tumors. It seems certain that changes in the function of the adrenal cortex are essential in both Cushing's syndrome and in virilism but this assumption may still be in keeping with our own theory that the hyaline change is associated with an increase of pituitary function.

In order to elucidate this problem it is necessary first to consider the source of the adrenal cortical hormones. Grollman (9) suggested that the androgens are derived from cells in a special 'androgenic zone' supposed to exist in the innermost layers of the cortex adjacent to the medulla, and this theory has been widely accepted. Zwemer, Wotton and Norkus (19), however, confirming the work of earlier investigators, showed that the cortical cells developed continuously from cells just beneath the capsule and that they differentiate progressively as they pass successively through the zona glomerulosa to ripen in the zona fasciculata and to degenerate in the zona reticularis where they finally disappear in the region adjacent to the medulla. This is confirmed by our findings that after hypophysectomy the adrenal cortex atrophies progressively from within outwards and no



histological abnormality occurs in the outermost zone (8). Thus it is probable that the cortical hormones are elaborated progressively into functionally completely developed steroids and then secreted by the cortical cells as they develop from without inwards. The androgenic steroids, which have no vital function, may be formed during this development or they may be breakdown products of the completely developed steroids but we find no evidence of a special androgenic zone in man.

It seems likely that in pathological lesions of the cortex, either tumors or hyperplasia, the constituent cells are at different stages of maturity in the different clinical disorders. The androgenic tumors and hyperplasia causing virilism would be composed mainly of either unripe or degenerating cortical cells, and the tumors and hyperplasia associated with Cushing's syndrome would be composed mainly of ripe cortical cells secreting fully formed corticosterones (3). The relationship of the pituitary gland to the different clinical disorders is demonstrated by the presence of hyaline basophilic cells in Cushing's syndrome but not in virilism or in cortical tumors unassociated with any endocrine manifestations. If these hyaline cells represent increased pituitary function as we have claimed, then it is clear that the ripening of the cortical cells in pathological conditions of the adrenal cortex is dependent on the increased production of the adrenotropic hormone by the pituitary gland. The final proof of this depends upon the satisfactory demonstration of an increased production of this hormone in Cushing's syndrome compared with that in virilism.

These theories would explain several perplexing observations. They would account for the very high androgen output in patients with virilism caused by adrenal cortical tumors composed of incompletely developed cells, producing mainly androgens and the lower output in patients with Cushing's syndrome caused by adrenal cortical tumors composed of completely developed or ripe cells which produce fully formed corticosterones. Secondly they would account for the remarkable unilateral atrophy of the adrenal cortex in patients with Cushing's syndrome associated with a tumor of the other adrenal. Such tumors produce a great excess of corticosterones (17) and it has been shown that certain of these hormones cause cortical atrophy in the experimental animal. Thus a greatly increased output of such substances by the tumor would result in atrophy of the remaining healthy adrenal cortex. Conversely, in women with virilism caused by an adrenal cortical tumor, the opposite adrenal is not atrophied. This is because the incompletely elaborated steroids which are being produced in excess do not cause cortical atrophy. Thirdly they would explain the occasional incidence of Addison's disease in children with adrenal cortical hyperplasia, often associated with pseudohermaphroditism or pubertas praecox (2, 15, 18). In these patients large amounts of androgens are produced to

the exclusion of fully developed corticosterones. Here is a relatively insufficient drive from the pituitary gland leading to the production of an inadequate amount of fully developed corticosterones. Fourthly they would account for the normal or only slightly increased output of androgens in patients with Cushing's syndrome without adrenal cortical tumor. In this condition the anterior pituitary gland acting as the instigator stimulates both adrenal cortices and this results in bilateral cortical hyperplasia and the production mainly of completely elaborated corticosterones.

There remains one outstanding question. Is it possible that in Cushing's syndrome associated with an adrenal cortical tumor, the pituitary gland acting as the primary instigator has stimulated the development of the adrenal neoplasm? The only evidence bearing on this question which has so far come to our attention is the finding of four cases of Cushing's syndrome associated with carcinoma of the pancreas (6). Three of these occurred in women aged thirty years or younger, an age at which carcinoma of the pancreas is very rare. We showed statistically that the chance of one such association is 0.015, of two, 0.000225 and of three, 0.000,003,375. If, as seems evident, this association is not fortuitous it follows that in Cushing's syndrome there is a propensity to tumor formation, a suggestion already made by McLetchie (12).

To return to the patient described in this paper, it is suggested that at the time of her Cushing's syndrome the pituitary drove her adrenal cortex to hyperplasia and perhaps to benign tumor formation with the production of an excess of completely formed corticosterones: that this hyperplasia or growth became arrested with the subsidence of pituitary stimulation, but at a later date malignant changes occurred which, in the absence of a marked pituitary drive, resulted in the production of incompletely formed corticosterones and a correspondingly increased output of androgens causing virilism.

#### SUMMARY

An unusual case is described of a woman who developed Cushing's syndrome with spontaneous remission. Subsequently she developed the clinical picture of virilism associated with an adrenal cortical carcinoma. Details of the necropsy are given. The endocrine disturbances responsible for Cushing's syndrome and virilism are discussed.

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# A RAPID METHOD FOR THE DETERMINATION OF TOTAL URINARY 17-KETOSTEROIDS

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**D**URING a metabolic study it was found necessary to determine total urinary 17-ketosteroids. Since many other urine analyses had to be performed, only a small volume of urine was available for 17-ketosteroid assay. In addition, the determinations had to be done within a limited period of time and on a large scale. Since the accepted methods (3, 7, 11, 12) required a minimum volume of approximately 500 ml. and a large amount of space and equipment, it was evident that modifications would have to be made.

Hydrolysis prior to extraction was introduced independently by two different groups of workers (4, 9) after the need for acidification of urine before extraction of capon-comb-growth-producing substance had been shown (1, 5, 6). Hydrolysis can be done prior to or simultaneously with extraction with a solvent. The latter procedure requires relatively expensive apparatus, a large volume of urine, carefully regulated conditions and much time.

Because of the ease of modification, independent hydrolysis lent itself more readily to the purposes of this investigation. However, since all the previous work was done on large volumes of urine, it was felt necessary to study the conditions for the hydrolysis and extraction of 17-ketosteroids in volumes of urine of 5 to 50 ml. These studies are reported in this paper.

## EXPERIMENTAL

*Effect of time, temperature and concentration of acid on hydrolysis.* Twenty ml. samples of normal male urine were hydrolyzed, with variations of time, temperature, and concentration of acid. Five ml. aliquots were assayed by the Holtorf-Koch modification (8) of the Zimmerman method. The results, as shown in Table 1, indicate that after heating for 10 minutes yields of 17-ketosteroids are low below 80°C. and above 100°C. Further,

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there is no advantage in using concentrations of hydrochloric acid above 30 per cent. The highest yields were obtained at 80°C. after 10 minutes or at 100°C. after 10 or 20 minutes with 30 per cent acid by volume. However, it was found that recovery of pure dehydroisoandrosterone added to urine prior to hydrolysis at 100°C. for 10 or 20 minutes varied from 82 per cent to 90 per cent; recovery of dehydroisoandrosterone after hydrolysis at 80°C. for 10 minutes varied from 94 per cent to 104 per cent. This indicated greater destruction of certain pure hormones at 100°C. than was present with hydrolysis at 80°C. It was also found that evaporation during

TABLE 1. EFFECT ON URINARY 17-KETOSTEROID YIELD\* OF VARYING (a), TIME OF HEATING AND (b), ACID CONCENTRATION OF URINE SAMPLES DURING HYDROLYSIS AT 80°C.

Spec. No.	Time Min-utes	Per Cent by Volume of Concentrated Hydrochloric Acid										
		0	10	20	25	30	35	40	50	60	80	100
1	10	—	—	22.7	—	26.9	24.1	—	—	—	—	—
1	20	—	—	23.8	—	25.2	23.0	—	—	—	—	—
1	60	—	—	23.0	—	23.2	13.5	—	—	—	—	—
2	10	0	4.2	27.4	—	29.1	—	—	27.9	26.1	29.7	27.9
3	10	—	—	—	14.6	16.0	15.5	15.2	—	—	—	—

\* Yields are given as milligram equivalents of dehydroisoandrosterone per 24 hours. Each result given is the average of 8 to 12 determinations.

hydrolysis at 100°C. was significantly greater than at 80°C. Therefore 80°C. was considered the most desirable temperature for hydrolysis.

*Extraction and washing.* Urine plus 30 per cent by volume<sup>1</sup> concentrated hydrochloric acid was heated at 80°C. for 10 minutes. Five ml. aliquots of hydrolyzed urine were extracted with petroleum ether, benzol, carbon tetrachloride or ethyl ether. Results are shown in Table 2. Petroleum ether yielded results which were much lower than those obtained with the other solvents. Ethyl ether was used as the solvent in this study because it extracted as much or more than the other solvents, did not form emulsions easily, and evaporated without excessive heating. Volumes of ether less than 20 ml. formed emulsions and larger volumes did not give increased yields. Rapid and vigorous shaking for 30 seconds was found adequate to obtain the intimate contact between solvent and solute necessary for the complete removal of 17-ketosteroids.

<sup>1</sup> Per cent by volume is defined as  $\frac{\text{Volume HCl used to hydrolyze urine}}{\text{Volume of urine being hydrolyzed}} \times 100$ .

One 10 ml. wash with 10 per cent sodium hydroxide followed by one wash with water was found sufficient to remove estrogens and other interfering substances. Additional washes with sodium hydroxide, sodium bicarbonate or water did not remove additional impurities.

TABLE 2. AMOUNT OF 17-KETOSTEROID EXTRACTED WITH DIFFERENT SOLVENTS FROM URINE HYDROLYZED AT 80°C. FOR 10 MINUTES

Results expressed as micrograms per 10 cc. sample

Spec. No.	Solvents			
	Ethyl Ether	Petroleum Ether	Carbon Tetrachloride	Benzol
1	38.0	16.0	26.5	28.2
2	25.0	15.3	25.5	25.3
3	39.7	29.7	38.0	39.7

#### METHOD

Based on the above experimental data the following procedure is suggested as being the most practical and accurate one for the determination of total urinary 17-ketosteroids.

*Reagents.* Absolute ethyl ether—reagent grade.

Absolute alcohol—commercial grade is usually suitable but it is best to run a Zimmerman test blank before using.

m-dinitrobenzene—Purify by heating in 10 per cent aqueous sodium hydroxide solution until melted. Decant while hot. Cool and wash twice with water. Add 95 per cent alcohol and dissolve by warming; keep temperatures below 50°C. Cool, add five volumes of distilled water and filter. Wash the precipitate twice with water and dry. Two per cent m-dinitrobenzene solution is made by dissolving 0.9 grams m-dinitrobenzene in 45 ml. absolute alcohol. Run a Zimmerman test blank on the reagent before using. It may be necessary to crystallize from alcohol again if blanks are high.

Potassium hydroxide—Five normal aqueous solutions of electrolytic grade KOH. Determine the normality with standard hydrochloric acid using methyl red as the indicator.

Sodium hydroxide—Solution of 10 Gm. reagent grade NaOH per 100 ml. water.

Concentrated hydrochloric acid—reagent grade. 37.5 per cent HCl. (by volume). In this paper the per cent of HCl by volume is defined as volumes of concentrated HCl per 100 volumes of urine.

It is advisable to run preliminary Zimmerman test reagent blanks to determine the purity of reagents.

*Procedure.* Place 10 ml. of urine and 3 ml. of concentrated HCl in a 125 ml. Erlenmeyer flask and stopper the flask with a Pyrex flathead stopper. Heat the flask in a water bath at 80°C. for 10 minutes, cool and transfer 5 ml. of the hydrolysate to a 125 ml. separatory funnel. Add 20 ml. ether and shake the funnel for 30 seconds. Remove the urine. Wash the ether once with 10 ml. of 10 per cent NaOH and once with 10 ml. of distilled water; shake for 10 seconds with each wash. Remove 5 ml. of ether, evaporate the 5 ml., and assay by means of the Zimmerman reaction (8). Similar aliquots can be taken for other assay methods such as the Pincus (10).

In our determinations, the Zimmerman was performed as follows. Add 0.2 ml. absolute alcohol, 0.2 ml. of the m-dinitrobenzene solution, and 0.3 ml. of the 5N KOH solution to the dried extract. Keep the solution in the dark in a water bath whose temperature is 27° for 90 minutes. After 90 minutes dilute the solution with 1 ml. of diluent; the diluent consists of three parts of absolute alcohol to one part of water. Read the diluted solution in a colorimeter<sup>2</sup> using a green filter.

Prepare the standard as directed above substituting 0.20 ml. of a solution of dehydroisoandrosterone in absolute alcohol (1.0 microgram hormone per 0.01 ml. alcohol) for the 0.20 ml. absolute alcohol and omitting the ether extract.

Prepare the urine blank as above, adding 0.20 ml. absolute alcohol in place of 0.20 m-dinitrobenzene solution.

Prepare method blanks by substituting water for urine in the hydrolysis and extraction; then proceed as above.

Prepare the Zimmerman reagent blanks as above, omitting the ether extract.

## RESULTS

*Reproducibility.* Duplicate samples of urine were assayed to determine the reproducibility of results. In over a hundred specimens the duplicates agreed within 5 per cent.

*Normals.* Twenty-four hour urine specimens from 35 normal young men between the ages of 20 and 30 were assayed for total urinary 17-ketosteroids. Determinations were made on 95 twenty-four hour specimens and the average 24 hour 17-ketosteroid excretion was found to be 16.9 mg. The amount excreted in 24 hours ranged from 10.0 mg. to 28.9 mg. with a mode falling between 16 and 17 mg. Seventy-nine per cent of the 24 hour speci-

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<sup>2</sup> The Drekter-Hoskins colorimeter was used in the present experiments.

mens assayed contained between 10.0 and 20.0 mg. of the 17-ketosteroid material.

*Recovery.* Varying amounts of androsterone, dehydroisoandrosterone and testosterone were added to urine.<sup>3</sup> The urine was then hydrolyzed at 80°C. for 10 minutes. Five ml. aliquots were then extracted with 20 ml. ethyl ether, washed with NaOH and water, and a portion of the ether extract evaporated and assayed. Results indicate that androsterone, dehydroisoandrosterone and testosterone can be recovered quantitatively.

### COMMENT

The proposed method is simpler than existing techniques. It has the advantage of requiring only small volumes of urine and ordinary inexpensive laboratory equipment. Furthermore, there is a considerable saving of time, which permits a complete determination to be done within three hours. Results are easily duplicated and agree well with those reported by Barnett et al. (2).

Results show that although slight increases in temperature and time of heating during hydrolysis do not affect results, it is advisable to maintain a minimum temperature of 80°C. for not less than 10 minutes. Poor recovery of dehydroisoandrosterone at 100°C. indicates the necessity for avoiding excessive heating. The amount of acid used should be 30 per cent by volume or more.

The method is flexible and can be easily adapted to the assay of volumes of urine smaller than 5 ml. or larger than 20 ml.

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# SPECIFIC RENAL FUNCTIONS IN HYPERTHYROIDISM AND MYXEDEMA

## EFFECTS OF TREATMENT

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THE abnormalities of metabolism and blood flow which characterize hyperthyroidism and myxedema have their effect on the kidneys. Urea clearance is depressed during myxedema although maximum specific gravity is not (2). But the kind and degree of renal functional changes in these diseases have not been analyzed by specific clearance methods. These enable the measurement of renal blood flow, glomerular filtration rate, tubular secretory capacity and, by calculation, renal vascular resistance. The purpose of this report is to describe observations before and after treatment in 4 patients, 2 suffering from hyperthyroidism and 2 from myxedema.

## PROCEDURES

Brief protocols of each patient are appended to this report. One of the hyperthyroid patients was treated by subtotal thyroidectomy (Dr. Goethe Link) and the other with thiouracil. The two patients suffering from myxedema were treated by oral administration of desiccated thyroid.

Routine observations include electrocardiography, cardiac teleroentgenography and frequent determinations of basal metabolic rate. The special studies of renal function were made shortly after admission to the hospital and repeated when the effects of treatment were established. These studies consisted in determination of the renal plasma clearances of diodrast and inulin and measurement of tubular secretory capacity for diodrast ( $Tm_D$ ). The procedures were substantially as described by Smith, Goldring and Chasis (9). The analytical methods were those of Corcoran and Page (4). The values reported are means of three satisfactory periods of urine collection and clearance measurement.

Plasma diodrast clearance is accepted as the equivalent of minimal renal plasma flow (RPF); the value  $RPF/1\text{-hematocrit}$  ratio is taken as the minimal rate of renal blood flow. Plasma inulin clearance measures the rate of glomerular filtration. The ratio of the clearances inulin/diodrast, known as filtration fraction (FF) expresses the proportion of water filtered

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through the glomerular capillaries from the plasma which perfuses them.  $Tm_D$  measures the maximum capacity of tubular cells to transfer diodrast from plasma to tubular fluid. This process involves the expenditure of cellular energy. In most situations, the function  $Tm_D$  can be taken as measuring the mass of functioning tissue. This can hardly be the case in diseases such as hyperthyroidism and myxedema in which tissue metabolism is seriously disturbed. Consequently, in this report, the function  $Tm_D$  is considered only as a measure of the metabolic activity of the tubular system concerned with the secretion of diodrast. The principles on which these interpretations are based are considered in detail elsewhere (5, 9). Renal vascular resistances were calculated by the method of Lampport (8). The  $R_A$  is taken as measuring resistance in the afferent vascular tract (aorta to end of glomerular capillaries) and  $R_E$  as measuring efferent resistance, principally that of the efferent arterioles.

## OBSERVATIONS

The data are summarized in Table 1.

TABLE 1. EFFECTS ON RENAL FUNCTION OF HYPERTHYROIDISM AND MYXEDEMA.  
RESULTS OF TREATMENT

Patient No.	Ante (A) or Post (P) Treatment	Renal Blood Flow cc. per min.	Glomerular Filtration Rate cc. per min.	Filtration Fraction	Tubular Secretory Capacity mg. D-I. per min.	$R_A$	$R_E$	Arterial Pressure mm. Hg	Basal Metabolic Rate %
1	A	810	112	0.23	35.4	.055	.018	150/55	+83
	P	795	119	0.27	32.3	.042	.018	120/88	+14
2	A	812	74	0.14	40	.062	.010	140/75	+58
	P	615	67	0.18	35.5	.079	.015	130/90	+19
3	A	509	55	0.17	11.1	.065	.018	105/80	-31
	P	600	101	0.27	37.3	.027	.026	100/70	+ 2
4	A*	448	33.6	0.11	13.6	.079	.016	94/88	-22
	P	617	80	0.18	32.7	.015	.019	90/46	+ 9

\* Had received 1 grain of thyroid extract daily for 5 days prior to this observation.

Legend: Effects on renal function, basal metabolic rate and arterial blood pressure of treatment of 2 patients (Nos. 1 and 2) suffering from hyperthyroidism and 2 (Nos. 3 and 4) suffering from myxedema. The rates of renal blood flow, glomerular filtration, secretory capacity are expressed respectively in cc. of plasma and mg. diodrast-iodine per minute per 1.73 square meters of surface area. Resistances ( $R_A$ =afferent,  $R_E$ =efferent) are calculated by the method of Lampport (8).

**Hyperthyroidism.** The absolute levels of the plasma clearances and of  $Tm_D$  were within normal limits at the first observation. In one patient (*No. 1*) restoration towards normal of the B.M.R. from plus 83 to plus 14 per cent by thyroidectomy did not significantly change the rates of renal blood flow and glomerular filtration, although the filtration fraction was somewhat increased. Renal vascular resistance was unchanged. Postoperatively,  $Tm_D$  was diminished by 10 per cent. In the other patient (*No. 2*) a decrease of the B.M.R. from plus 58 to plus 19 per cent was associated with a moderate decrease in renal blood flow, and increases in filtration fraction and diastolic arterial pressure. These changes were accompanied by an increase in renal vascular resistance. The pattern of renal function is consistent with that of essential hypertension.  $Tm_D$  was decreased by about 12 per cent.

**Myxedema.** An increase in basal metabolism from minus 31 to plus 2 per cent in one patient (*No. 3*) was associated with an 18 per cent increase in renal blood flow, an 83 per cent increase in glomerular filtration rate and an increase in the filtration fraction. The function most affected was  $Tm_D$  which increased 236 per cent. In the second patient of this pair (*No. 4*) a change in B.M.R. from minus 22 to plus 9 per cent was accompanied by a 38 per cent increase in renal blood flow, a greater increase in filtration rate (+138 per cent) and a 140 per cent increase in  $Tm_D$ . It should be noted that this patient had been under treatment with desiccated thyroid for five days at the time of the first observation.

In spite of the restoration towards normal of the basal metabolism in these two patients and the associated clinical improvement, the absolute and relative levels of renal blood flow had not returned to normal at the time of the second observation. In both also, treatment resulted in a decrease of the renal vascular resistance  $R_A$  which measures resistance from the aorta to the end of the glomerular capillaries.

#### DISCUSSION

**Hyperthyroidism.** Surprisingly, the rates of renal blood flow, glomerular filtration and tubular secretory capacity ( $Tm_D$ ) are not increased in proportion to the increase of basal metabolic rate. The changes ( $-10$ ,  $-12$  per cent) in  $Tm_D$  postoperatively probably reflect decreases in renal cellular metabolism. However, the degree of these changes just exceeds the limits of reproducibility of the procedure (5). Hyperthyroidism, at least in these two patients, was not associated with renal vasodilation comparable to that which occurs in the extra-renal circulation, nor does it greatly alter tubular secretory capacity. Experimentally, administration of thyroid extract to normal dogs increases plasma diodrast clearance by only 5 per cent, while  $Tm_D$  increases by nearly 50 per cent (7). An increase in  $Tm_D$  of

115 per cent has been observed in a dog given thyroxin (6).  $Tm_D$  is evidently more altered by experimental than it is by clinical hyperthyroidism.

**Myxedema.** The sluggishness of function in this condition is reflected in decreases below normal levels of renal blood flow, glomerular filtration and  $Tm_D$ . Treatment with desiccated thyroid increases renal blood flow, roughly in proportion to the increase in basal metabolism. Glomerular filtration rate is disproportionately increased, apparently as the result of vasodilation in the afferent vascular tract, possibly of the afferent arterioles. The changes of urea clearance found in myxedema by Beaumont and Robertson (2) are thus seen to reflect changes in glomerular filtration. Treatment also causes a disproportionately great increase in  $Tm_D$ . This effect is especially noteworthy when the relative changes of  $Tm_D$  and B.M.R. in myxedema are compared with those in hyperthyroidism.

The increase in  $Tm_D$  observed as the result of treatment of myxedema is probably not an expression of increased tissue metabolism resulting from stimulation by thyroid hormone. On the one hand, the increase greatly exceeds the degree of concurrent changes in B.M.R. and, on the other, the changes of  $Tm_D$  in hyperthyroidism are comparatively slight. Alternatively, the decrease of  $Tm_D$  found in myxedema and its restoration towards normal on treatment, may reflect the depressing effect of myxedema and the stimulating effect of treatment on the eosinophilic cells of the anterior hypophysis. It has been suggested (1) that these cells exert a trophic influence on renal tubular secretory function. Hypophysectomy in dogs greatly decreases  $Tm_D$  (12) and treatment with anterior lobe extracts increases this function (7). Thyroidectomy in rats decreases the size and number of eosinophilic cells, the change being proportional to the reduction in thyroid tissue (11). It is therefore suggested that the depression of renal function present in some patients with myxedema reflects in part a functional depression of the anterior hypophysis rather than a direct lack of thyroïdal hormone.

#### SUMMARY AND CONCLUSIONS

Observations of specific renal functions in 2 patients suffering from hyperthyroidism, 1 treated by subtotal thyroidectomy and the other with thiouracil, indicate that this condition is not associated with a significant renal vasodilation or with an increase in tubular secretory capacity for diodrast which is proportionate to the elevation of the basal metabolic rate.

Observations in 2 patients suffering from myxedema and treated with desiccated thyroid demonstrate before treatment reductions of renal blood flow, glomerular filtration and tubular secretory capacity. Treatment results in restoration of these functions towards normal. The changes thus induced in renal blood flow and glomerular filtration rate reflect a

decrease in afferent vascular resistance. The depression of tubular secretory capacity in myxedema is excessive in relation to the reduction of basal metabolic rate in this condition and to the changes observed in this function during hyperthyroidism. It is suggested that the renal functional depression present in myxedema is in part a reflection of depression of anterior hypophyseal function rather than a direct renal lack of thyroidal hormone.

### PROTOCOLS

*Patient No. 1. J. R.*, a woman aged 67, showed symptoms of hyperthyroidism, (weakness, weight loss, nervousness, tremor, eye signs and elevated basal metabolic rate) in 1939, at which time she was treated by administration of iodine, vitamins, high caloric diet and irradiation of the thyroid gland. Symptoms recurred in 1943 and, after partially effective medical treatment with renewed irradiation of the gland, operation revealed colloid and fetal adenoma. The after-treatment observations recorded in Table 1 were made three weeks after subtotal thyroidectomy.

*No. 2. M. W.*, a woman aged 72, complained of weakness, palpitation, nervousness, and weight loss increasing for one year. Examination revealed tremor, exophthalmos, positive eye signs and increased perspiration with heat intolerance. The initial observation was made November 26, 1943. She was placed on thiouracil 0.2 Gm. twice daily and the dosage gradually increased to 0.4 Gm. three times daily, on which dosage she was maintained. The after-treatment observation was made March 3, 1944. High caloric diet with vitamin supplements was given throughout the treatment period.

*No. 3. M. T.*, a woman, aged 43, had noted weakness, cold intolerance, transitory psychosis and gain in weight over one year. Examination showed the typical appearance of myxedema, confirmed by electrocardiogram and basal metabolic rates. Treatment was begun with  $\frac{1}{2}$  grain daily of desiccated thyroid, increased to 3 grains and later decreased to 2 grains daily on which latter dose she was maintained at the time of post-treatment observation. This observation was made 3 months after beginning treatment.

*No. 4. C. B.*, a man aged 47, had myxedema, diagnosed from weakness, slowness of thought, speech and action, cold intolerance, appearance and basal metabolic rate (-40 per cent) in 1938, at which time he was treated with desiccated thyroid. In June, 1943, he was brought to the hospital in toxic delirium with paranoid trends. His appearance was typical of myxedema. The history, obtained from his wife, revealed that he had ceased taking thyroid extract some weeks before admission. Because of his greatly depressed state and inability to cooperate or retain food, treatment was begun at once, the first observations of basal metabolic rate and renal function being made after five days administration of thyroid extract, 1 grain daily. His condition gradually improved and the psychosis disappeared under treatment. The post-treatment observation was made two months after his admission.

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# REPORT OF A CASE SHOWING CONGENITAL DEFECTS, SHORT STATURE, RETARDED SEXUAL DEVELOPMENT AND NO URINARY GONADOTROPINS

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THE following case is presented because we believe that any differences and variations from the so-called "clear cut case" should be recorded in the literature.

## CASE REPORT

In December 1943, a Polish girl, S. S., 16½ years old, came to the Children's Endocrine Clinic with the complaint that she had not grown in height since the age of twelve, and that she had never menstruated nor had she developed any secondary sexual characteristics.

The patient was the fifth in a family of six children. Her mother and four sisters all had a normal menstrual history and apparently were sexually well developed. There was no history of any gross endocrine dyscrasia in any of the immediate family. The patient had had the ordinary childhood diseases. She had been alert mentally and showed good progress at school. Her infantile developmental history pertaining to holding head erect, teething, sitting up, walking and talking, was normal. The patient thought that her linear growth from birth to the age of twelve years seemed to keep pace with that of her friends of similar age. At the age of twelve, however, she noted and was told that her growth had become retarded and soon it became stunted. She also noted that she failed to develop any sizable amount of breast tissue, that pubic or axillary hairs did not appear, and that she never menstruated.

### *Physical Examination:*

At the time of the first examination on December 27, 1943, she presented a short stature, obesity, a webbed neck and a lack of secondary sexual characteristics (Fig. 1). There was an absence of breast tissue, and an absence of pubic hairs, except for the presence of a few on the labia majora. The labia minora were small and hypoplastic. By rectal examination the body of the uterus could not be delineated, but a small hypoplastic cervix could be felt. In addition, it was found that the teeth were carious and showed malpositions and malocclusions. The eyes revealed a slight exophthalmos, a normal fundus and no abnormal ocular muscle palsies. The ring finger of her right hand was congenitally foreshortened. Her weight was 130 pounds and her linear height 54 inches. She presented eunuchoid skeletal proportions.

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*Laboratory Data:*

The fasting blood sugar was 94 mg. per 100 cc. and the total cholesterol was 300 mg. per 100 cc. of blood. There were no abnormalities in a casual urine examination. The Wassermann test was negative and the basal metabolic rate was minus 26 per cent. A sugar tolerance test (Exton-Rose) showed a fasting blood sugar of 95 mg.; the first sample thereafter was 119 mg. and the second sample was 96 mg. (This is indicative of a hypoglycemic curve.)

Roentgenograms of her skull showed no evidence of increased intracranial pressure;



FIG. 1. S. S., age  $16\frac{1}{2}$  years. Note webbed neck, absence of breast development and pubic hair.

the sella turcica was within normal limits in size and there were no erosions of the floor or clinoid processes. Her cervical spine showed no fusion of the intervertebral discs (a Klippel-Feil syndrome was ruled out). There was a congenitally elongated second cervical vertebra which showed a tendency to forward subluxation. Roentgen ray examination of her carpus showed a delay in the closure of the epiphyses of the phalanges and the other epiphyses; a rudimentary small fourth metacarpal bone was noted. The tibia and fibula showed no evidence of periosteal changes. Roentgenograms of the chest showed no abnormal finding of the heart or lungs; there was no coarctation of the aorta.

Hormonal assays were made of the patient's urine for urinary gonadotropins, estrogens and 17-ketosteroids. Urinary gonadotropins (hypophyseal follicle-stimulating hormone, or F.S.H.) were tested according to the modification of Zondek's technique (4) by Kurzrok and Miller. This test is a qualitative one, and if positive, indicates the presence of at least 100 m.u. of F.S.H. per liter. A negative response to this test is found in normal females during the reproductive age. A positive test would indicate that there were increased urinary pituitary gonadotropins. According to the method outlined above, which was used on two different occasions and performed in two different laboratories, our patient showed no perceptible urinary pituitary gonadotropins either before or after three years of hormonal therapy. Urinary estrogens were measured according to the acid hydrolysis method of Smith and Smith (6). Here, results would indicate that normal women should excrete 4 to 20 R.U. in 24 hours. Our patient showed only slight traces of estrone. Neutral 17-ketosteroids in the urine were determined by the colorimetric method of Sachs and Kurzrok (3). Normal values by this method range from 2 to 5.6 mg. in 24 hours in the adult female. Our patient showed a normal value of 3 mg. in 24 hours.

#### *Treatment:*

Varied hormonal therapy,<sup>1</sup> which included estrogens, progesterone and thyroid, was instituted. Three years of treatment produced no perceptible changes either in height, sexual development or in urinary gonadotropins.

### DISCUSSION

Since 1938, when Turner (7) reported 7 cases with the "Syndrome of infantilism, congenital webbed neck and cubitus valgus," there have been accumulating in the literature quite a few reports of cases like ours. Varney, Kenyon and Koch (8) in 1942 called it "Association of short stature, retarded sexual development and high urinary gonadotropin titers in women: ovarian dwarfism." Albright and his colleagues (1) in 1942 called it "A syndrome characterized by primary ovarian insufficiency and decreased stature." Schneider and McCullagh (5) in 1943 used the term "Turner's syndrome"; Wilkins and Fleischmann (9) in 1933 called it "Ovarian Agenesis," and only recently E. B. del Castillo et al. (2) called it a "Syndrome of rudimentary ovaries."

From their reports it was apparent that all were describing cases characterized principally by shortness in stature, hypoplastic genitals, primary amenorrhea, an absence of secondary sexual characteristics, congenital abnormalities of skeletal structure, diminished 17-ketosteroids, an insufficiency of estrogens, and increased pituitary follicle-stimulating hormone (F.S.H.) or urinary gonadotropins.

Our case differed in that there was found to be an absence of follicle-stimulating hormone, a trace of estrone, and normal 17-ketosteroids, as shown by tests made at two laboratories on two different occasions. Wheth-

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<sup>1</sup> We are indebted to Dr. Stoner of the Schering Corporation for a liberal supply of Progynon B, Proluton and Prometron.

er this places our case in a subgroup of the syndrome described by the other investigators remains to be seen and depends upon whether other such cases with similar assays are found.

In spite of the variations in our case which may place it in a subgroup, we, like some of the other investigators, think that the cause of this syndrome is genetic. Further, we believe that one of the congenital defects is an involvement of the gonads to a marked degree and a moderate involvement of the anterior pituitary gland. The marked involvement of the ovaries would account for the genital defects and open epiphyses; and the moderate involvement of the anterior pituitary gland would account for the stunting instead of dwarfism. It would perhaps also account for the absence of urinary gonadotropins.

### SUMMARY

This report covers the case of a 16½ year old girl with stunted growth, primary amenorrhea, no secondary sexual characteristics, obesity, a delay in osseous status as evidenced by open epiphyses, and in addition such characteristic congenital defects as webbed neck and cubitus valgus.

Hormonal assays showed only traces of estrone, normal 17-ketosteroids and an absence of urinary gonadotropins (F.S.H.) rather than the usual absence of estrone, low 17-ketosteroids and an excess of urinary gonadotropins.

If, in the future, other cases with findings such as ours occur, it is likely that these might be considered a subgroup of this syndrome, which was originally described by Turner.

We think that the cause for the syndrome in our case is in all likelihood genetic. Further, we believe that among the congenital defects manifested, one is an involvement of the gonads to a marked degree, and another a moderate involvement of the anterior pituitary gland which would account for the endocrine symptoms.

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# THE TREATMENT OF THYROTOXICOSIS WITH AMINOTHIAZOLE—RESULTS IN TWENTY-THREE CASES

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IT is our purpose in this report to present the results of treatment with aminothiazole in 23 cases of thyrotoxicosis. This compound (Fig. 1) appears to have been first used clinically in the treatment of thyrotoxicosis in France in 1943 (9), following the observation of goitrogenesis and improvement of thyrotoxic symptoms among workers in chemical plants who were exposed to the compound. Perrault and Bovet (10) and other French writers (3, 4, 6) have reported satisfactory results from the use of aminothiazole in thyrotoxicosis, but their data are not susceptible to critical

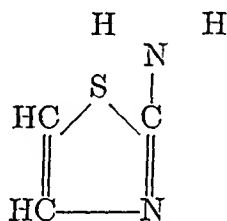


FIG. 1. Aminothiazole.

analysis. The only report of its clinical use which we have found in the American literature is that of Williams (11), who employed it in 9 cases. One patient developed jaundice and another dermatitis, fever and arthritis while under treatment. Williams lists this compound as of low clinical potency and desirability among antithyroid drugs. The goitrogenic and antithyroid action of aminothiazole in the rat was found by Astwood and others (2) to compare with that of thiouracil in the ratio of 0.15 to 1.00. Astwood (1) also reported its antithyroid activity in the rat to be less than that of thiourea. Like many other goitrogenic compounds it inhibits, in a manner not yet clear, the synthesis of thyroid hormone within the gland.

Our 23 cases fall into two groups—9 patients who were given aminothiazole in preparation for thyroidectomy, and 14 in whom treatment was begun with the hope of producing a prolonged or permanent remission without operation. Data relating to the first group are presented in Tables 1 and 2. It will be seen that withdrawal of therapy was necessary because

of toxic or sensitivity reaction in 5 cases before any effect was noted. One of the 4 patients who received aminothiazole during the entire preoperative period was also given iodine for 13 days prior to thyroidectomy. This iodination was necessary because the patient was becoming increasingly disturbed emotionally as a result of her failure to enter a remission after 38 days on aminothiazole. No significant change was noted in the size or consistency of the thyroid glands of these 4 patients. At operation the glands were somewhat firm rather than friable, and hemorrhage was not

TABLE 1. DATA RELATING TO PATIENTS TREATED WITH AMINOTHIAZOLE

	Preoperative Use	Prolonged Use
a. Sex		
Males	2	2
Females	7	12
b. Goiter type		
Diffuse	7	14
Nodular	2	0
c. Previously treated	3	7
d. Basal metabolic rate (Initial range)	+25 to +69 per cent	+23 to +65 per cent
e. Daily dosage	0.6 gm.	Initially 0.6 gm. Maintenance 0.1-0.2 gm.

troublesome. The postoperative course was uneventful in these patients. Histologic sections from the 2 toxic diffuse goiters showed considerable variation in different areas. Rather marked increase in colloid content and flattening of acinar epithelium, comparable to the changes produced by iodine, appeared interspersed among areas showing little colloid and marked epithelial proliferation of columnar type. Neither of these patients had received iodine.

Similar variation from moderate hyperplasia of acinar epithelium with diminished colloid content, to marked increase in colloid acinar content with flattened cuboidal epithelial cells was observed in the two toxic nodular goiters. The goiter of one of these patients, who had received iodine in addition to aminothiazole for the last 13 days of the preoperative period, also showed marked fibrosis and areas of calcification and old and recent hemorrhage. The histologic changes in these 4 goiters differ from those observed in patients treated with thiouracil, in that the degree of hyper-

plasia and vascular engorgement was less intense and uniform in the former.

Eight of our patients were subjected to thyroidectomy following iodination, at intervals varying from 9 to 45 days after aminothiazole had been stopped because of untoward reactions. Aminothiazole had been given to these patients for periods varying from 8 to 30 days. One patient

TABLE 2. RESULTS OF PREOPERATIVE USE OF AMINOTHIAZOLE (9 CASES)

Patients satisfactorily prepared	4
Preoperative period	17, 27, 51, 92 days
Patients showing unsatisfactory response (Reactions)	5

was operated upon 8 months after withdrawal of aminothiazole. The histologic appearance of the thyroid glands of these patients showed no significant difference from that usually seen after iodine therapy in toxic goiter, with the possible exception that vacuolization of the colloid was rather prominent.

Data relating to the 14 patients in whom therapy was undertaken in an effort to produce prolonged or permanent remission are presented in Tables 1 and 3. It will be noted that aminothiazole had to be withdrawn in 7 cases because of untoward reactions. Four of these patients were subsequently subjected to thyroidectomy; 2 are still under treatment with

TABLE 3. DATA RELATING TO PROLONGED TREATMENT WITH AMINOTHIAZOLE (14 Cases)

	Minimum	Maximum	Average
Onset of remission (days)	30	75	51
Duration of treatment (months)	2	12	5.9
Sustained remission			
a. Still under treatment			4
b. After treatment was discontinued			3
Unsatisfactory responses (reactions)			7

propylthiouracil, and one who is recovering from an hepato-toxic reaction is receiving no antithyroid treatment at the time of writing. Three of the 7 patients who showed satisfactory response have remained in remission for 8, 8, and 2 months respectively after the drug was stopped. They were treated for 4, 2, and  $3\frac{1}{2}$  months respectively. One patient showed a definite decrease in the size of her goiter after  $3\frac{1}{2}$  months of treatment. Two pa-

tients (both 13 year old girls) have shown appreciable increase in the size of their thyroid glands after 3 and 4½ months of therapy respectively. No significant change in exophthalmos has been observed in any patient during or after treatment. One patient, a man aged 65 with auricular fibrillation and congestive heart failure, has been kept under satisfactory control with reversal to normal cardiac rhythm and freedom from congestive signs for 7½ months on a daily maintenance dose varying from 0.05 Gm. to 0.2 Gm.

Our usual initial dose of aminothiazole was 0.6 Gm. daily given in three divided doses for adults, and 0.4 Gm. daily for the two children. During preoperative preparation the initial daily dose was not reduced. The basal

TABLE 4. TOXIC AND SENSITIVITY REACTIONS TO AMINOTHIAZOLE

Patient	Duration of Treatment Prior to Onset	Daily Dose at Onset Gm.	Total Dose at Onset Gm.	Type of Reaction	Duration of Reaction
D.C.	9 days	0.6	5.4	Fever	3 days
S.M.	2 months	0.4	30.4	Hepatitis with jaundice	2 weeks
C.A.-G.	15 days	0.6	9.0	Fever, urticaria arthralgia, subcutaneous nodules	acute—1 month chronic—7 months
D.B.W.	9 days	0.6	5.4	Fever, nausea, vomiting	2 days
L.M.	8 days	0.6	4.8	Chills, fever, macular rash	1 month
M.C.	9 days	0.6	5.4	Fever	3 days
J.F.	8 days	0.6	4.8	Urticaria, macular rash	1 week
B.D.	3 months	0.6	38.8	Hepatitis with jaundice	2 weeks
D.L.	11 days	0.6	6.6	Arthralgia, nausea, vomiting	1 week
W.P.	22 days	0.6	13.2	Generalized lymphadenopathy, fever	1 week
D.W.	28 days	0.6	16.8	Arthralgia, fever, urticaria	1 week
H.L.	36 days	0.6	21.6	Intermittent pruritis	10 days

metabolic rate was determined once weekly in hospital patients and every two weeks in outpatients. In patients receiving prolonged treatment the daily dose was gradually reduced as remission occurred; the ultimate daily maintenance dose varied from 0.05 Gm. to 0.2 Gm.

There may have been some correlation between the initial basal metabolic rate and the time required for remission to appear. Thus, in 3 patients with initial basal metabolic rates above plus 56 per cent, remission occurred in an average of 59 days; in 4 patients with initial basal metabolic rates between plus 44 and plus 45 per cent remission appeared in an average of 53 days; and in 3 patients with initial basal metabolic rates of plus 38 per cent or below, the average time required for remission was 42.5 days.

Data relating to toxic or sensitivity reactions are presented in Table 4. Untoward reactions forcing interruption of therapy occurred in 12 cases, or 52 per cent. These reactions, in descending order of frequency, included: (1) fever with malaise, sometimes accompanied by nausea and vomiting;



(2) skin eruptions, either urticarial or maculopapular, with severe pruritis; (3) arthralgia, with or without fever and malaise; (4) jaundice with evidence of hepatic cell damage as demonstrated by liver function studies; (5) pruritis without skin eruption; (6) generalized lymphadenopathy. Reactions appeared in from 8 to 90 days after treatment was instituted, the average time being 25.5 days. Reactions appeared in 15 days or less in 7 of the 12 patients. Jaundice appeared in 2 patients, 60 and 90 days respectively after beginning treatment, with respective total doses of 30.8 Gm. and 38.8 gm. of aminothiazole. Both of these patients were more jaundiced than ill, although evidence of hepatic damage was obtained by liver function studies in each case. Arthralgia occurred in 3 cases. In 2, it was of brief duration and involved the hands, wrists, elbows and shoulders. The third patient was of unusual interest as the following protocol will show:

### CASE REPORT

Mrs. C. A-G., a white married woman, aged 30, was admitted to the Surgical Division on October 30, 1946, with a diagnosis of toxic diffuse goiter. Her symptoms, which were typically those of thyrotoxicosis, had begun in May 1943, following the neonatal death of her first child. The previous medical history was irrelevant. On admission she presented a typical picture of toxic diffuse goiter with slight exophthalmos and moderate diffuse enlargement of the thyroid. The basal metabolic rate was plus 69 per cent. Preoperative medication was begun with aminothiazole 0.2 Gm. three times daily. Evidence of remission of the thyrotoxicosis was only slight during the first two weeks. On the fifteenth day of treatment an urticarial eruption appeared over the extensor surfaces of the knees and elbows. This spread rapidly until it involved most of the trunk, extremities and face, and was accompanied by pruritus and recurrent bouts of angioneurotic edema. Aminothiazole was discontinued at the onset of the urticaria. Two days later severe pain and swelling appeared in the knees, wrists, and elbows, accompanied by a rise in temperature to 103.6° F., extreme malaise and collapse. Subsequently the shoulders, hands, and jaws were involved and reddish tender indurated areas appeared over both arms and legs. These varied in diameter from about 1 to 4 cm. and subsided slowly over a period of 2 to 3 weeks. The patient was very ill and movement of the involved joints caused excruciating pain. The total leucocyte count did not rise above 10,000 per cubic mm. and the eosinophiles varied from 0 to 6 per cent. Cultures from throat, urine and blood stream revealed no pathogenic organisms. Irregular and gradually subsiding fever continued with slow improvement in joint and cutaneous manifestations for about 23 days, at which time the patient was fairly comfortable except for some residual pain and stiffness in knees, shoulders, elbows and wrists. Iodinization was begun 9 days after the onset of the urticaria and 25 days later a subtotal thyroidectomy was performed with an uneventful postoperative course. The patient has been followed for 6 months since operation. There has been no recurrence of the thyrotoxicosis. Stiffness, slight swelling and intermittent pain have recurred with decreasing frequency and severity in the previously affected joints. Minor urticarial outbreaks with slight swelling of the face and eyelids have recurred occasionally. The sedimentation rate of the red cells has remained within normal limits throughout. Roentgen rays of the affected joints have shown nothing abnormal. The entire episode seems to be attributable to the aminothiazole and the persistence of symptoms is noteworthy.

The reactions involving fever, malaise, gastrointestinal disturbances, and maculopapular skin eruptions subsided within 7 days except in the case of one patient who showed persistent fever and grippe-like symptoms for two weeks. The total leucocyte count declined by 1000 cells or more per cubic mm. in 8 patients during treatment. The lowest leucocyte count was 3900 per cubic mm. Neutropenia did not occur in any case.

Morgans (7) reported the following reactions in 13 patients treated with aminothiazole:

Drug fever	6
Rashes	5
Adenopathy	1
Jaundice	2
Nausea and vomiting	3

Perrault (8) reported the following incidence of reactions in 129 cases: digestive disturbances, including jaundice (5 per cent); oliguria with lumbar pain (incidence not stated); fever and malaise (7 per cent).

Perrault and Bovet (10) mention the occurrence of a pinkish discoloration of serum and urine by aminothiazole but we did not observe this in any of our patients.

Eckfeld, Seifter et al. (5) were able to recover only traces of aminothiazole from the blood and urine of 4 of our patients who had taken the drug for 5, 6, 7 and 7 weeks respectively in doses of 0.8 Gm., 0.2 Gm., 0.6 Gm., and 0.4 Gm. daily. They had a similar experience with dogs which had but recently received large doses of the drug, and concluded that attempts to adjust dosage by determining blood levels were futile. They produced fatal poisoning in dogs, with severe hepatic damage, both by single doses of 250 mg. per kg. and repeated doses of 60 to 80 mg. per kg. of body weight.

It is possible that a distinction should be drawn between toxic and sensitivity reactions in those patients who exhibited intolerance to aminothiazole. The transitory arthralgias, adenopathy, fever and cutaneous manifestations may well have been phenomena of sensitivity. The objective evidence of hepatic damage found in our 2 jaundiced patients, however, suggests a direct toxic effect on the liver. The prolonged joint symptoms described in our patient, C. A-G., suggest that the drug may in this case have initiated a series of events which should be considered more than simply phenomena of drug sensitivity.

It is apparent that the results of treatment of thyrotoxicosis with aminothiazole in the doses employed in our small series of cases do not compare favorably with those attainable either by subtotal thyroidectomy or by such antithyroid compounds as thiouracil and its derivatives. The high incidence of untoward reactions alone would seem to render this substance impractical for clinical use. In view of Astwood's report (2) that the anti-

thyroid action of aminothiazole in the rat was only about  $\frac{1}{6}$  that of thiouracil, it may be asked whether we should have employed larger doses. The occurrence of undesirable reactions in 52 per cent of our patients, including 2 with hepatic damage, would appear to be sufficient answer to this objection.

### SUMMARY AND CONCLUSIONS

1. The results of the administration of aminothiazole<sup>1</sup> to 23 patients with thyrotoxicosis have been reported.

2. Toxic or sensitivity reactions sufficient to warrant withdrawal of treatment occurred in 12 patients (52 per cent). Two patients became jaundiced with evidence of hepatic damage. One patient showed an unusually prolonged reaction characterized by arthralgia and urticaria recurring for 7 months.

3. Three patients were successfully prepared for thyroidectomy with aminothiazole alone. At operation the consistency of their thyroid glands was firmer, and the glands less friable, than is usually the case in patients prepared with thiouracil or its derivatives.

4. Three patients have remained in remission for from 2 to 8 months following withdrawal of the drug.

5. Aminothiazole appears to possess no advantages over thiouracil compounds in the treatment of thyrotoxicosis, and its marked tendency to produce toxic or sensitivity reactions would appear to preclude its clinical use.

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# IODINE-LACK THEORY AND ENDEMIC GOITER

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THE article on lack of iodine and endemic goiter by Greenwald (8) and the heated commentaries that followed its publication prompt me to comment on the contents of this publication and its criticisms and to discuss briefly the problem in question.

In 1932, I published a monograph of 60 pages, concerning goiter and the iodine-lack theory (26) which critically surveyed the world literature on this problem and contained a series of original investigations into the iodization of some goitrous and nongoitrous districts in Germany. Strangely enough, Greenwald did not refer to this publication which is based on most of the material he himself used and in which I came to some of the same conclusions about the iodine-lack theory.

Greenwald has, I believe, confounded the issues considerably. On the other hand, his critics have dealt with his article in a highly emotional manner. As the inconsistencies which I noted when revising the then available evidence for and against the iodine-deficiency theory are not unlike some of Greenwald's observations, I feel entitled to take part in the discussion which has arisen out of his recent publication.

Greenwald has been unfortunate enough to connect the question as to whether lack of iodine is the cause of endemic goiter with the theory and the results of *iodine prophylaxis* of which he says that "in some cases there has been no effect at all, or even an increase in the incidence" (of goiter). He also mentions that the "changes in the virulence in the disease" may have been mistaken for the results of the prophylactic use of iodine which "has not reduced the incidence of new goiters to zero." Although the literature on the etiology of endemic goiter reveals the complexity of the problem, the general opinion about the value of iodine prophylaxis appears to be uniform, and the published results which, by now, extend over more than two decenniums, leave no doubt that the administration of iodine represents an important preventive method against endemic goiter. It seems therefore unjustified and unwise to use criticisms against the value of iodine prophylaxis as an argument against the iodine-lack theory, because neither good nor bad results obtained with the prophylactic administration of iodine are a proof for or against the theory that endemic goiter is caused by lack of iodine. Also, this simple logic has unfortunately not been fully realized by the champions of iodine prophylaxis. Many of them have at the

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same time become the most ardent protagonists of the iodine-lack theory, while other workers in this field are still of the opinion that "simple iodine-lack is no doubt not the whole story" (Iodine and Goitre. *Lancet*, 1: 754, May 31, 1947). Kimball (16) does not agree. The summary of his paper, read at the Third International Goiter Conference, begins as follows:

"We have definitely proven that:

(1). Simple or endemic goiter is a deficiency disease and this deficiency is iodine in our food and drink . . .

(2). The addition of an exceedingly small amount of iodine to our food in endemic goiter regions prevents goiter . . . "

It should be clear to everyone that 2) can be true even if 1) is a fallacy, and the scientists on councils and committees who are occupied with the introduction of iodine prophylaxis of endemic goiter need not be alarmed, should the iodine-lack theory prove to be wrong. Many discoveries in medicine were based on wrong theories which have since proved useful or even revolutionizing. This has not prevented anyone in the past from revising the bases of such discoveries and from uncovering any erroneous propositions, and we hope that, in spite of interference by agencies and the daily press, the same attitude will also prevail in the future, should a similar occasion arise.

The second point which demands clarification is the iodine-lack theory itself. Here again, Greenwald's conclusion that endemic goiter is not due to a lack of iodine does not seem precise enough, as it does not distinguish between the two aspects of the iodine-lack theory. Chatin (4) who first introduced it on a scientific basis has formulated it as follows:

1. Le goitre et le crétinisme sont inconnus dans les contrées normalement iodurées (Goiter and cretinism are unknown in districts with a normal iodization).
2. Ces maladies se montrent quand la proportion de l'iode diminue (These diseases appear when the proportion of iodine diminishes).

According to Chatin's conception the iodine-lack theory, of endemic goiter means that goiter and cretinism are due to an insufficient iodization of the district (= primary exogenous lack of iodine), a fact he thought to have proved by iodine analyses of water, food, soil, air, derived from goitrous localities. A commission, appointed by the French Academy to investigate Chatin's claims, failed to confirm his results in 1852, and the theory fell into disrepute until McClendon et al. (21) in America (1923) and von Fellenberg (6) in Switzerland (1923-26) revived it on the strength of new iodine assays by means of a more modern microtechnique.

On the other hand, students of thyroid physiology and pathology who have investigated the relationship between iodine and thyroid function since Baumann's discovery that iodine is a normal constituent of the thyroid (1896), have been led to the conclusion that goiter formation and io-

dine metabolism are linked together. Since it has been shown that goiter represents a compensatory hypertrophy, the hypothesis was put forward that "the immediate cause of goiter is failure of the thyroid gland to obtain an adequate supply of iodine" (Harington (9)) or, as Marine (18) formulated it earlier, that "the immediate cause of thyroid enlargement is a relative or an absolute deficiency of iodine." Thus, the iodine-lack theory contains two distinct problems, and the questions that have to be answered are:

1) Is the immediate cause of goiter an inadequate supply of iodine to the thyroid gland?

2) If so, are goitrous districts inadequately iodinated and is this the cause of a deficient iodine supply to the thyroid gland?

Both questions have been critically discussed by Greenwald and answered in the negative, but I feel that the distinction between them has not been made clear enough, especially as over half of his paper is devoted to question 2). His conclusion that endemic goiter is not due to lack of iodine may mean that lack of iodine does not lead to goiter formation, or that deficient iodination of the locality does not, or that both do not. Much depends on the precise answer to these questions.

Regarding the first, which represents the iodine-lack theory in its widest aspect, I think most workers will agree that lack of iodine may play a part in the pathogenesis of goiter, but that we still do not understand the whole problem of goiter formation and are unable to say with certainty how important iodine deficiency is and what other factors are involved. The occurrence of sporadic as well as endemic goiter and the difference in structure between the goiters in mountainous and non-mountainous regions suggest that there may be more than one causative factor. The more recent discovery of goitrogenic factors in plants and of the goitrogenic action of synthetic thiourea derivatives point to a possible role of goitrogenic substances as primary agents which may, however, owe their goitrogenic activity to an interference with the utilization of iodine by the thyroid gland. Animal experiments with iodine-poor diets and attempts to prevent this dietary goiter by the addition of small doses of iodine have not given conclusive results. Goiter can be experimentally produced by different other factors, such as exposure to cold, high protein or fat diet, starvation, and excess of calcium. Iodine-poor diets seem to produce goiter more easily when there is an excess of calcium in the diet; iodine deficiency alone is often followed by atrophy of the gland (Hellwig (11) Thompson (25) etc.). All these observations throw doubts on the correctness of hypothesis 1). The discovery of definite goitrogenic substances which act regardless of the amount of iodine intake, though perhaps by interfering with the utilization of iodine by the thyroid, is especially important. There is little evi-

dence for Kimball's conclusion that it has been "definitely proven that simple or endemic goiter is a deficiency disease and this deficiency is iodine in our food and drink." I venture to say that there is a certain amount of evidence indicating that this is not so. In any case, we need further evidence before hypothesis 1) can be regarded as a fact.

A great deal of work has been done to prove the correctness of hypothesis 2) which represents an older and more specific form of the iodine-lack hypothesis. Here Greenwald's analysis of the facts seems to me worth considering and follows lines similar to my own (1932). The reasons against the validity of this form of the iodine-lack theory are partly methodical, partly based on the actual results gained from iodine estimations of soil, water and food that were meant to show the existence of an inverse correlation between goiter incidence and iodination of the locality. The conclusions which I drew in the quoted monograph ((26) p. 426) seem to me as correct today as at the time they were written. It would be sufficient to quote them again without further comment, but on account of the highly controversial nature of the problem it seems more appropriate to give a brief account of the reasons which led to their adoption. Details have to be looked up in the publication itself and can also be found in Greenwald's paper.

1) There can be no doubt that the methods used for iodine analyses of foodstuffs etc. were not only unreliable but that the error exceeded any permissible error of a biochemical microanalytical method. I worked with von Fellenberg's original method (6) about which criticism was raised by the author himself as late as seven years after the introduction of the method (quoted by Ucko (26) p. 375). On the other hand, von Fellenberg was unable to obtain satisfactory results with McClendon's method (21) and also criticized various modifications of his own method which were introduced later on. In fact, it is no exaggeration to say that the greater part of the published results on iodine estimations in various materials may be methodically wrong, and it appears even doubtful whether the error is constant enough to compare the results obtained by the same author in his different analyses. To give an example of the situation I have tabulated the figures which different authors have obtained in analyzing the iodine content of bread and potatoes in goitrous and nongoitrous districts of various parts of the world. (See Table 1.) The figures vary between 8 and 115 micrograms for bread and between 8 and 85 micrograms for potatoes in non-goitrous localities alone. If one compares the figures for goitrous and non-goitrous districts with each other, one can easily see that they overlap considerably and that a decisive difference is only evident if the figures of the same author are compared. The same is true if the figures for other foodstuffs, soil, water, etc. are compared in this way.



TABLE 1. IODINE CONTENT OF BREAD AND POTATOES IN DIFFERENT DISTRICTS  
(micrograms per kilogram)

District	Bread		Potatoes		Authors
	Non-goitrous	Goitrous	Non-goitrous	Goitrous	
Argentina					
Buenos Aires	—		32		Mazzocco (19)
Salta		—		12-20	Mazzocco (19)
New Zealand	12		22		Hercus (12) (13)
Switzerland			31	17; 35	Fellenberg (6)
La Chaux-de-Fonds	16		18		Fellenberg (6)
Signau (cf. p. 7)		8		11	Fellenberg (6)
Germany					
Bavaria	75-92	70-76	28	20	Bleyer (2)
Palatinate	102-115		34		Bleyer (2)
Holstein	—		32		Bleyer (2)
Danzig	0		85		{ Glimm (7)
East Prussia	50		15		{ Isenbruch (15)
Greifswald	19		7-9		Ucko (26)
Kiel	8		10-13		Ucko (26)
North Friesian Islands	—		15		Ucko (26)
Berlin	28		9-14		Ucko (26)
Kassel Town	61		6-17		Ucko (26)
Kassel County	43	32-39	13	11-13	Ucko (26)
Merseburg County	65	22	8	14	Ucko (26)
Kottbus		43		15	Ucko (26)
Glatz County		27-32		2-18	Ucko (26)
Hungary	44	25	15	5	Bodnar & Straub (3)

2) On reading the evidence for an inverse relationship between goiter incidence and iodination of the locality, serious doubts arise also about the estimation of goiter incidence. Such statistics have been collected sometimes by the observer himself, sometimes by local authorities, by commissions, etc, and the criteria used vary considerably. As an example of the unreliability of such statistics it may be recalled that one of von Fellenberg's classical findings in favor of the iodine-lack theory was the difference in La Chaux-de-Fonds and Signau in Switzerland, the former be-

lieved to be goiter-free, the latter goitrous. However, Stiner (24) reported in 1927 that goiter occurs in over 50 per cent of school children and in 21 per cent of 20-year-old males in La Chaux-de-Fonds, as compared with 28 per cent of the same age group in Signau. Both places seem therefore to have nearly the same goiter incidence and had to be put into the same group in Stiner's goiter map of Switzerland. Such happenings throw serious doubts on the reliability of the iodine estimations and show how careful one should be in drawing conclusions from available data.

3) There seems to be no doubt that certain regions in which goiter occurs endemically, are rich in iodine (1, 14, 17, 20) and that endemic foci occur in districts near the sea where a high iodination of the locality definitely exists. If endemic goiter were a "deficiency disease" caused by insufficient iodine supply and if this were the only possible cause of endemic goiter, such occurrences would be completely impossible.

4) Although a number of workers have found less iodine in foodstuffs, soil, water, etc. in goitrous than in non-goitrous regions, a comparison of figures does not show that the lower the iodination of the district, the higher the incidence of goiter. This inverse relationship should, however, be unmistakable if goiter was due to deficient iodine intake because of insufficient iodination of the district. I therefore agree with Greenwald, that a primary exogenous iodine deficiency has not yet been sufficiently proved. Attempts have been made to calculate the daily iodine intake by estimating the amount of iodine present in a theoretical daily menu. A few figures from different countries will suffice to show that these figures are also not convincing and cannot prove an exogenous iodine deficiency in goitrous regions. Thus, von Fellenberg estimated the daily iodine consumption in Switzerland to be 60 micrograms in non-goitrous, 20 micrograms in goitrous districts; Lunde's (17) corresponding figures for Norway are 35 and 20 micrograms; I have found from 31 to 53 micrograms in non-goitrous and minor endemic districts of Germany, and Reith (23) found an average daily intake of 150 micrograms in Holland where endemic foci occur in spite of an iodine intake  $2\frac{1}{2}$  times that of Switzerland. The possibility of a secondary deficiency remains, though conclusive data are likewise lacking. Secondary deficiency could be produced by a variety of exogenous factors, such as one-sided nutrition, loss of iodine by inadequate preparation of food or certain environmental factors, or it may be caused by endogenous factors such as impaired absorption, increased demands, interference with the utilization of iodine (this may be produced by specific exogenous goitrogenic substances). The cause of endemic goiter, however, must be one which acts generally on all the inhabitants and even on the animals in the district, as it has been shown that the average weight of the thyroid glands

even of non-goitrous individuals is higher throughout life than in non-goitrous districts.

5) It is impossible to say that Chatin's hypothesis that an exogenous lack of iodine is the cause of endemic goiter has been proved. This does not mean that lack of iodine cannot be the cause of goiter, although there seems to be reason to assume that it is not the only cause. It is here that the controversies start, and Greenwald's analysis would perhaps have been received with more positive criticism if he had analyzed the different aspects of the iodine-lack theory instead of refuting it indiscriminately. The whole problem can only be solved by a further analysis of the iodination in definitely goitrous and non-goitrous districts with a technique that fulfils the demands of a reliable microanalytical method. In the same way, further investigations into the iodine excretion of goitrous and non-goitrous individuals are necessary, whereby the question has to be examined again whether the normal organism is in the state of iodine equilibrium. Greenwald's publication contains a full critical survey of the results obtained so far from estimation of the iodine excretion. Previously published figures by Clark & Adams (5) for Massachusetts, Lunde (17) for Norway, and von Fellenberg (6) for Switzerland, present problems similar to those quoted by Greenwald.

It is hoped that this brief analysis of the available evidence for and against the iodine-lack hypothesis of endemic goiter will help to clarify the matter, to pave the way for further research, and also to show that the iodine prophylaxis of endemic goiter is not affected in any way by etiological theories.

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# LETTERS TO THE EDITOR

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TO THE EDITOR:

## THE INTRANASAL USE OF POSTERIOR PITUITARY POWDER IN THE TREATMENT OF DIABETES INSIPIDUS: A REMINDER

THE present limited supply of aqueous solutions of posterior pituitary has become a matter of concern for those patients with diabetes insipidus whose therapy has been based on the injection of this preparation. This circumstance has prompted us to call the attention of those who might be unfamiliar with it to an alternative procedure, namely, the intranasal insufflation of posterior pituitary powder U.S.P., of which the antidiuretic efficacy has been well established and which has the additional advantages of greater convenience and economy.

The initial observations as to the effectiveness of this procedure were made by André and Lucie Choay in 1924,<sup>1</sup> shortly after Blumgart's demonstration of the therapeutic value of the intranasal application of aqueous solutions of posterior pituitary.<sup>2</sup> Their experience has been confirmed by many others, and reviewed and supplemented with additional favorable results by Smith.<sup>3</sup> In spite of such general agreement as to its merits, the intranasal use of posterior pituitary powder has not been as extensive as its effectiveness, convenience and economy would warrant.

A brief summary is given in Table 1 of our own findings as to the relative efficiency of the powder given intranasally, the aqueous extracts subcutaneously, and pitressin tannate in oil intramuscularly, in 7 patients with diabetes insipidus. With the exception of patient *N.B.* whose course of therapy was too brief to permit a satisfactory evaluation, all have been well regulated by the intranasal insufflation of the powder. This is administered by a De Vilbiss spray, number 44, which is small enough to be carried in the pocket or purse and delivers approximately 5 mg. of powder with

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<sup>1</sup> Choay, A. and Choay, L. Traitement du diabète insipide par des inhalations d'extrait de lobe postérieur d'hypophyse. *Rev. neurol.* 40: 267-269 (Feb. 7, 1924).

<sup>2</sup> Blumgart, H. L. The antidiuretic effect of pituitary extract applied intranasally in a case of diabetes insipidus. *Arch. Int. Med.* 29: 508-514 (April 1922).

<sup>3</sup> Smith, F. M. Diabetes insipidus. Treatment by intranasal insufflation of posterior lobe pituitary powder. *J.A.M.A.* 102: 660-664 (March 3, 1934).

each jet. This method enables the patient to administer the required amount of antidiuretic therapy at appropriate intervals without the incon-

TABLE 1

Patient, Age, Sex	Pitressin Subcutaneously		Pitressin Tannate in Oil		Posterior Pituitary Powder by Nasal Insufflation	
	Dosage	Therapeutic Response	Dosage	Thera- peutic Re- sponse	Dosage	Thera- peutic Re- sponse
T.K. 48 M	10 U b.i.d. 20 U b.i.d.	Good, for 12 years then poor Poor			1 jet* each nostril t.i.d.	Good
R.M. 63 M			5 U q. 36 hours	Fair	1 jet each nostril 4 i.d.	Good
M.F. 12 F	10 U b.i.d.	Fair			1 jet each nostril t.i.d.	Good
S.S. 16 F			5 U	Poor	1 jet each nostril t.i.d.	Good
N.B. 7 M			5 U q.d. 4 U b.i.d.	Poor Good	1 jet each nostril q. 3 hours	Poor†
M.T. 40 F	10 U 6 i.d.	Poor			2 jets each nostril 4 i.d.	Good
J.W. 15 M					1 jet each nostril t.i.d.	Good

\* Each jet equivalent to approximately 5 mg. posterior pituitary powder.

† Therapy for only one week at this dosage level; then stopped.

venience entailed in the use of a hypodermic syringe. There have been no complaints of significant nasal irritation, despite the uninterrupted use of the powder, in some instances, for as long as 2 years.

The cost of therapy with posterior pituitary powder is from  $\frac{1}{8}$  to  $\frac{1}{8}$  that of parenteral preparations at current retail prices.

ANNE C. CARTER  
EPHRAIM SHORR  
September 2, 1947

*Department of Medicine  
Cornell University Medical College and  
The New York Hospital  
New York City*

TO THE EDITOR:

### FATAL JAUNDICE RESULTING FROM THIOURACIL

Fatal cases of agranulocytosis as well as non-fatal cases of jaundice resulting from the use of thiouracil have already been reported. The following is perhaps the first reported case of fatal jaundice resulting from the use of that drug.

#### REPORT OF CASE

G. N., a Spanish businessman, aged 48, had been ill for eight months, complaining of nervousness, palpitation, tremor and marked loss of weight. Examination revealed a diffusely enlarged, non-nodular thyroid and moderate exophthalmos. He also suffered from duodenal ulcer. The patient stated that for some time he had been taking Lugol's solution but without beneficial results. His weight had dropped to 59 kg. The pulse rate was 100, blood pressure 140/75, and the basal metabolic rate +37 per cent. Thiouracil was prescribed and the patient was put on a dosage of 0.6 Gm. daily. After ten days of thiouracil therapy the pulse was 78 and the basal metabolic rate +17.2 per cent. Dosage was repeated for ten more days at the end of which time the pulse was 74 and basal metabolic rate +10 per cent. Inasmuch as the patient's condition had improved a great deal and his weight had increased to 63.2 kg., thiouracil dosage was cut down to 0.4 Gm. daily. White blood cell counts during treatment were normal. Two weeks later symptoms of intense jaundice appeared. Thiouracil was immediately discontinued and treatment with glucose and B complex started at once, with diet and rest. There was not, however, the slightest improvement and the patient died eight days later. No autopsy was allowed but the clinical signs pointed toward acute yellow atrophy of the liver.

#### COMMENT

The dosage given in this case is no higher than that usually prescribed at present. This confirms the opinion that toxic reactions are not dependent upon the amount of the drug administered. Notwithstanding its recent use, thiouracil has been responsible for some fatalities. The utmost care is required in its administration and it is the author's opinion that it should be employed only in special cases.

LUCIANO DÉCOURT  
August 21, 1947

*From the Butantan Institute,  
São Paulo, Brazil*

# ANNOUNCEMENT OF THE 1948 MEETING OF THE ASSOCIA- TION FOR THE STUDY OF INTERNAL SECRETIONS

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The Thirtieth Annual Meeting of the Association for the Study of Internal Secretions will be held in the Palmer House, Chicago, Illinois, June 18 and 19, 1948.

The scientific sessions will be held in the Red Lacquer Room and registration will be on the fourth floor just outside the Red Lacquer Room. The Annual Dinner will be held in the same room on Friday, June 18th at 7 p. m. and will be preceded by a cocktail party, the location of which will be announced later.

All members of the Association who plan to attend the Thirtieth Meeting are urged to make their reservations at once with the Palmer House, stating the time of arrival and how long they plan to remain in Chicago.

Nominations for awards and fellowships of the Association must be in the Secretary-Treasurer's office by March 15, 1948.

Those wishing to present papers should send title and four copies of a comprehensive abstract of not more than 200 words and suitable for publication in the program to Dr. C. N. H. Long, 333 Cedar Street, New Haven, Conn., not later than March 15, 1948.





# Announcement of Awards and Fellowship of the Association

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## *Nominations for Awards*

Three awards for meritorious work in endocrinology will be given at the next annual meeting of the Association. A special committee of five members of the Association chooses the recipients of these Awards, subject to ratification by the Council, and each member of the Association has the privilege of making one nomination for each award.

Nominations for the Awards should be made on special application forms which may be obtained from the Secretary, Dr. Henry H. Turner, 1200 North Walker Street, Oklahoma City 3, Oklahoma. All nominations, accompanied by a statement of the importance of the nominee's contributions to endocrinology and a bibliography of his most important papers with reprints if possible, should be sent to Dr. Turner's office not later than March 15, 1948.

### THE E. R. SQUIBB AND SONS AWARD

The E. R. Squibb and Sons Award of \$1,000.00 was established in 1939. It was given in 1940 to Dr. George W. Corner; in 1941 to Dr. Philip E. Smith; in 1942 to Dr. Fred C. Koch; in 1944 to Dr. Edward A. Doisy; in 1945 to Dr. E. C. Kendall; in 1946 to Dr. Carl G. Hartman; in 1947 to Drs. Carl F. and Gerty T. Cori. No award was made in 1943. No age or special limitation is stipulated by the donor of the award.

### THE CIBA AWARD

The Ciba Award, established in 1942, is given in recognition of the meritorious accomplishment of an investigator, not over 35 years of age, in the field of clinical or pre-clinical endocrinology. In 1944 the Award was given to Dr. E. B. Astwood; in 1945 to Dr. Jane Anne Russell; in 1946 to Dr. Martin M. Hoffman and in 1947 to Dr. Choh Hao Li. The Award is for \$1,200.00. If within two years of the date of the Award, the recipient chooses to use it to aid in working in a laboratory other than the one in which he normally is located, the Award will be increased to \$1,800.00.

### THE AYERST, McKENNA & HARRISON FELLOWSHIP

The first award of the Ayerst, McKenna & Harrison Fellowship was given to Dr. Samuel Dvoskin in 1947. The fellowship was founded in order to encourage investigation in the field of endocrinology rather than as an award

for work done. The amount of the fellowship is \$2,500.00 annually. The nominee must possess the degree of Doctor of Philosophy or Doctor of Medicine or their equivalent. It is suggested that no restriction be placed on age, but that preference be given to applicants who have recently completed the requirements for their Ph. D. or M. D. degree. The nominee must present evidence of scientific ability as attested by studies completed or in progress and/or the recommendation of responsible individuals; submit a program of proposed study; indicate one or more institutions where the proposed program will be carried out; submit statement of approval from the investigators with whom he proposes to conduct his research; serve full time if awarded a fellowship. A small amount of time (10 to 15 per cent) may be allotted for course work or for participation in teaching, the latter purely on a voluntary basis.

## AMERICAN ASSOCIATION FOR THE STUDY OF GOITER

### VAN METER PRIZE AWARD

The American Association for the Study of Goiter again offers the Van Meter Prize Award of Three Hundred Dollars and two honorable mentions for the best essays submitted concerning original work on problems related to the thyroid gland. The Award will be made at the annual meeting of the Association which will be held in Toronto, Canada, May 6th, 7th, 8th, 1948 providing essays of sufficient merit are presented in competition.

The competing essays may cover either clinical or research investigations; should not exceed three thousand words in length; must be presented in English; and a typewritten double spaced copy sent to the corresponding secretary, Dr. T. C. Davison, 207 Doctors Building, Atlanta 3, Georgia not later than February 1st, 1948. The committee, who will review the manuscripts, is composed of men well qualified to judge the merits of the competing essays.

A place will be reserved on the program of the annual meeting for presentation of the Prize Award Essay by the author if it is possible for him to attend. The essay will be published in the annual Proceedings of the Association. This will not prevent its further publication, however, in any Journal selected by the author.

T. C. DAVISON,  
*Corresponding Secretary*

# Postgraduate Course in Endocrinology

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The Postgraduate Committee of THE ASSOCIATION FOR THE STUDY OF INTERNAL SECRETIONS, under authority of its Council, announces a course of lectures and demonstrations in CLINICAL ENDOCRINOLOGY to be held in LOS ANGELES at the BILTMORE HOTEL, FEBRUARY 23 to 28, 1948, inclusive.

The faculty will consist of the most prominent investigators and clinical endocrinologists in the various branches of the medical sciences in the United States and Canada.

It is the intent of the Committee that this course be a practical one of interest and value to both the GENERAL PRACTITIONER AND THE SPECIALIST.

A fee of \$100 will be charged for the entire course and the attendance will be limited to 100. Registration will be in the order of checks received and will close on February 1, 1948. Should there be an insufficient number of applicants to warrant the course, the registration fee will be immediately refunded in full.

Please make your application on your letterhead and forward, together with your check payable to THE ASSOCIATION FOR THE STUDY OF INTERNAL SECRETIONS, to DR. E. KOST SHELTON, CHAIRMAN of the POSTGRADUATE COMMITTEE, 921 WESTWOOD BOULEVARD, LOS ANGELES 24, CALIFORNIA.

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Since satisfactory hotel accommodations are still difficult to procure on short notice in Los Angeles, especially during the winter season, it is suggested that all applicants MAKE THEIR RESERVATIONS EARLY.

SOME LARGE HOTELS IN THE METROPOLITAN AREA OF  
LOS ANGELES:

Alexandria  
Ambassador  
Biltmore

Chapman Park  
Gaylord  
Hayward

Lankershim  
Mayflower  
Town House

# INDEX TO VOLUME 7

## AUTHOR INDEX

\* Original article.

† Abstract of paper presented at the Twenty-ninth Annual Meeting of The Association for the Study of Internal Secretions.

Other items are abstracts of articles which appeared in current medical journals.

- A** BARBANEL, A. R.: Correlation of basal body temperature curves with endometrial biopsy, 451†
- ALBRIGHT, F.: Polyostotic fibrous dysplasia: A defense of the entity, 307\*
- , see FORRES, A. P., 264\*
- , see TALBOT, N. B., 331\*
- ALMY, T. P. and SHORR, E.: Disappearance of diabetes mellitus associated with acromegaly following acute mastoiditis and basilar meningitis, 455†
- APPELMAN, D. H., see DORFF, G. B., 807\*
- ARGONZ, J., see CASTILLO, E. B. DEL, 385\*
- ASPER, S. P., JR., see WILLIAMS, R. H., 462†
- ASTWOOD, E. B. and VANDERLAAN, W. P.: Treatment of hyperthyroidism with propylthiouracil, 304
- AYRE, J. E.; CHEVALIER, P. M., and AYRE, W. B.: A comparative study of vaginal and cervical cornification in human subjects, 749\*
- and FOOTE, W. R.: Granulosa-cell tumor with pregnancy following removal, 605
- AYRE, W. B., see AYRE, J. E., 749\*
- B** AEZ, S., see ZWEIFACH, B. W., 460†
- BAKER, B. L. and LEEK, J. H.: The relationship of the parathyroid glands to the action of estrogen on bone, 383
- BAKST, H., see KISSIN, M., 152\*
- BALLINGER, J.: The co-existence of hyperthyroidism and prepuberal eunuchoidism in a male, 566\*
- BALZE, F. A. DE LA: Influencia del hipertiroidismo sobre la densidad y contenido en proteínas del plasma, 75
- , see CASTILLO, E. B. DEL, 385\*
- , see CASTILLO, E. B. DEL, 493\*
- BARKER, H. B.: A detailed report on the weights and weight losses of twenty-four men in Santo Tomas internment camp, 71
- BARKER, W. W., see ROGERS, H. M., 383
- BARMAN, J. M. and PORCILE, E.: A simple method for assuring accuracy in determination of oxygen consumption by the Benedict-Roth apparatus, 304
- BARTCZAK, E., see DREKTER, I. J., 795\*
- BARTELMEZ, G. W., see ROSSMAN, I., 609
- BATES, R. W., see COHEN, H., 452†, 701\*
- BAUER, J. and BELT, E.: Paroxysmal hypertension with concomitant swelling of the thyroid due to pheochromocytoma of the right adrenal gland, 30\*
- BAUMGOLD, D., see SELEY, A. D., 451†
- BAYLOR, N.: Tables for predicting height from the skeletal age and present height, 473
- BEIERWALTES, W. H. and STURGIS, C. C.: Complications following the administration of thiouracil, 305
- BELT, E., see BAUER, J., 30\*
- BENDA, C. E. and BIXBY, E. M.: Urinary excretion of 17-ketosteroids in various conditions of oligophrenia correlated with some autopsy observations, 503\*
- BENGA, R. S., see MORROW, A. G., 608
- BERGNER, G. E., see DEANE, H. W., 457†
- BIANCO, J. J. and FAVORITE, G. O.: Granulosa-cell tumor of the ovary, 605
- BIXBY, E. M., see BENDA, C. E., 503\*
- BLACK, R., see ZONDEK, B., 519\*
- BLOCK, F. B.: Ovarian Tumors, 226
- BOLGER, H., see FISHER, A. E., 231
- BOROVSKI, M. L.: The role of the nervous system in autotransplantation of the thyroid, 75
- BOURNE, A.: Endoerines in gynecology, 384
- BRADBURY, J. T.: A simplified method for the estimation of sodium, 72
- BRANDBURY, J. T., see BROWN, W. E., 450†
- BRECKLER, A., see FLEISCHMANN, W., 468†
- BRIGGS, A. P.: Some observations on severe diabetic ketosis treated with glucose and insulin, 229
- BRIGGS, G. M. and LILLIE, R. J.: Perosis caused by feeding high levels of thiouracil, 76
- BROWN, W. E.; BRANDBURY, J. T., and JENNINGS, A. F.: Experimental alteration of the human ovarian cycle by estrogen, 450†
- BROWNE, J. S. L.; HENRY, J. S., and VENNING, E. H.: Studies in corpus luteum function, 446†
- , see VENNING, E. H., 79\*, 460†, 729\*
- BROWNELL, K. A., see HARTMAN, F. A., 461†

- BUCHWALD, E., *see* SHIPLEY, R. A., 70
- BURGESS, A. M.: Myxedema controlled by thyroid extract for fifty-two years: report of a case, 76
- CALKINS, E. and HOWARD, J. E.: Bilateral-familial phaeochromocytomata with paroxysmal hypertension: successful surgical removal of tumors in two cases, with discussion of certain diagnostic procedures and physiological considerations, 475\*
- , *see* LERMAN, J., 77
- CAMP, J. D., *see* SIGLIN, I. S., 433\*
- CANTAROW, A., *see* PASCHKIS, K. E., 102\*, 466†
- , *see* RAKOFF, A. E., 448†, 688\*
- CAPRIGLIONE, L. and SCHERMANN, J. Head trauma causing reactivation of thyrotoxicosis, appearance of diabetes mellitus, amenorrhea and melonoderma, 303
- CARPENTER, T. M., *see* ROOT, H. F., 472
- CARR, E. A.: A rapid bedside test for the detection of hypoglycemia, 72
- CARTER, A. C. and ROBBINS, J.: The use of hypertonic saline infusions in the differential diagnosis of diabetes insipidus and psychogenic polydipsia, 464†, 753\*
- and SHORR, E.: (Letter to Editor). The intranasal use of posterior pituitary powder in the treatment of diabetes insipidus: a reminder, 828\*
- CASTILLO, E. B. DEL; BALZE, F. A. DE LA, and ARGONZ, J.: Syndrome of rudimentary ovaries with estrogenic insufficiency and increase in gonadotropins, 385\*
- ; GALLI-MAININI, C., FINOCHIETTO, R., LUCHETTI, S. E. and STAFFIERI, J. J. El tiouracilo como tratamiento preoperatorio del hipertiroidismo en 37 pacientes, 76
- ; TRABUCCO, A., and BALZE, F. A. DE LA: Syndrome produced by absence of the germinal epithelium without impairment of the Sertoli or Leydig cells, 493\*
- CHARIPPER, H. A., *see* GORDON, A. S., 306
- CHESLEY, J. C.; COSGROVE, S. A., and PREECE, J.: Hydatidiform mole, with special reference to recurrence and associated eclampsia, 605
- CHESNER, C.: Hemochromatosis. Review of literature and presentation of a case without pigmentation or diabetes, 72
- CHEVALIER, P. M., *see* AYRE, J. E., 749\*
- CIARAMELLI, L. C., *see* MASON, H. L., 458†
- COATES, C. W., *see* WEISMAN, A. I., 289\*
- COHEN, E. J.: An evaluation of the urethral smear as an index of androgenic deficiency in the male, 186\*
- COHEN, H. and BATES, R. W.: A simple quantitative colorimetric method for estrogenic steroids, 701\*
- : A simple quantitative colorimetric test for estrogens, 452†
- COHN, G. M., *see* FINKLER, R. S., 455†
- COLLINS, J. A., JR., *see* STAINSBY, W. J., 779
- CONN, J. W. and MATHEWS, K. P.: Addison's disease in the Negro, 69
- CONNOR, J. F. and REYNOLDS, F. W.: The two-dose dextrose tolerance test in the diagnosis of diabetes mellitus, 229
- CORCORAN, A. C. and PAGE, I. H.: Specific renal functions in hyperthyroidism and myxedema, 801\*
- CORI, CARL F. and CORI, GERTY T.: Recipients of E. R. Squibb & Sons award of the Association, 223
- COSGROVE, S. A., *see* CHESLEY, J. C., 605
- COVOLO, G. C. and WEST, R.: The activity of arginase in red blood cells, 325\*
- CRAFTS, R. C.: The effects of iron, copper and thyroxine on the anemia induced by hypophysectomy in the adult female rat, 376
- CROOKE, A. C.: The endocrine disorders associated with Cushing's syndrome and virilism, 787\*
- CURTIS, A. H.: Another case of arrhenoblastoma, 606
- : The origin of adrenal-like tumor of the ovary, 605
- CURTIS, G. M., *see* PUPPEL, I. D., 380
- CURTIS, L. E., *see* LISSER, H., 665\*
- DAUGHADAY, W. H.; JAFFE, H., and WILLIAMS, R. H.: Chemical assay of urine for adrenocortical hormones in endocrine and non-endocrine diseases, 454†
- , *see* WILLIAMS, R. H., 462†
- DAVISON, R. A.; KOETS, P., and KUZELL, W. C.: Excretion of 17-ketosteroids in ankylosing spondylarthritis and in rheumatoid arthritis. A preliminary report, 201\*
- DAX, E. C.; SMITH, E. J. R., and REITMAN, F.: Adrenalectomy in mental disorder, 533
- DEANE, H. W. and BERGNER, G. E.: Chemical and cytochemical studies of the rat's adrenal cortex following the administration of pituitary adrenocorticotrophic hormone, 457†
- DÉCOURT, L.: (Letter to Editor). Fatal jaundice resulting from thiouracil, 830\*
- DELORY, G. E.: Seminal fluid acid phosphatase in sterility, 604
- DOBYNS, B. M.: Exophthalmos and tissue changes in the guinea pig following administration of the thyroid stimulating hormone of the pituitary gland, 305

- and HAINES, S. F.: Changes in the prominence of the eyes in various thyroid states, 306
- DOLGER, H., *see* HERZSTEIN, J., 727
- DONALDSON, E. C., *see* FORBES, A. P., 264\*
- DORFF, G. B.; APPELMAN, D. H., and LIVERSON, A.: Report of a case showing congenital defects, short stature, retarded sexual development and no urinary gonadotropins, 807\*
- DORFMAN, R. I., *see* SHIPLEY, R. A., 70
- DOUGLAS, J. W., *see* WEED, J. C., 455†, 741\*
- DREKTER, I. J.; PEARSON, S., BARTCZAK, E. and MCGAVACK, T. H.: A rapid method for the determination of total urinary 17-ketosteroids, 795\*
- ; PEARSON, S., and MCGAVACK, T. H.: A rapid method for the determination of urinary "17-ketosteroids," 451†
- DUMM, R. M. and SHIPLEY, R. A.: The simple estimation of blood ketones in diabetic acidosis, 230
- DVOSKIN, S.: Recipient of Ayerst, McKenna and Harrison fellowship of the Association, 223
- EATON, L. M., *see* SIGLIN, I. S., 433\*
- EDMONDSON, H. A.; MARTIN, H. E., and EVANS, N.: Necrosis of renal papillae and acute pyelonephritis in diabetes mellitus, 471
- ELLIOTT, J. E. and PEARSON, P. B.: A direct photoelectric method for the determination of serum calcium, 73
- ENGELHARDT, H. T. and MELVIN, J. P., JR.: The management of diabetes mellitus during pregnancy, 230
- ERSHOFF, B. H.: Effects of liver feeding on growth and ovarian development in the hyperthyroid rat, 532
- ESCAMILLA, R. F., *see* LISSER, H., 665\*
- EVANS, H. M., *see* MASON, H. L., 458†
- EVANS, N., *see* EDMONDSON, H. A., 471
- EVERETT, H. S., *see* JONES, G. S., 607
- FARBER, E. P.: The induction of labor with methergine: preliminary report, 606
- FARRIS, E. J.: A test for determining the time of ovulation and conception in women, 606
- : The time of ovulation in the monkey, 384
- FAVORITE, G. O., *see* BIANCO, J. J., 605
- FEINER, M., *see* KRICHESKY, B., 448†
- FELDMAN, F.; ROBERTS, J. B.; SUSSELMAN, S., and LIPETZ, B.: Coincidence of diabetes mellitus and hypopituitarism, 376
- FINCH, C. A., *see* HILLS, A. G., 458†
- FINERTY, J. C., *see* KUPPERMAN, H. S., 447†
- FINKELSTEIN, G., *see* GORDON, A. S., 306
- FINKLER, R. S.: Male hypogonadism treated by sublingual methyltestosterone, 293\*
- : Social and psychological readjustment of a pseudohermaphrodite under endocrine therapy, 456†
- and COHN, G. M.: Testosterone in a case of polyostotic fibrous dysplasia, 455†
- FINOCHIETTO, R., *see* CASTILLO, E. B. DEL, 76
- FISHER, A. E. and BOLGER, H.: Behavior and psychologic problems of young diabetic patients, 231
- FLEISCHMANN, W. and BRECKLER, A.: Mitotic activity and wound healing in the corneal epithelium of rats treated with thiouracil, 468†
- , *see* WILKINS, L., 381
- FOLDES, F. F. and MURPHY, A. J.: Distribution of cholesterol, cholesterol esters and phospholipid phosphorus in blood in thyroid disease, 76
- FOOTE, E. C. and JONES, C. E. S.: An evaluation of the Hogben pregnancy test, 606
- FOOTE, W. R., *see* AYRE, J. E., 605
- FORBES, A. P.; DONALDSON, E. C.; REIFENSTEIN, E. C., JR., and ALBRIGHT, F.: The effect of trauma and disease on the urinary 17-ketosteroid excretion in man, 264\*
- FORSHAM, P. H.; PRUNTY, F. T. G., and THORN, G. W.: Urinary uric acid-creatinine ratio following administration of pituitary adrenocorticotrophic hormone (ACTH) as a simple test for adrenal cortical function, 459†
- , *see* HILLS, A. G., 458†
- , *see* THORN, G. W., 459†
- FOSS, H. L. and KLINGER, H. M.: Hyperthyroidism without goiter, 377
- FOSTER, W. C., *see* MCCLENDON, J. F., 468†, 714\*
- FREED, S. C.: Diethylstilbestrol dipalmitate in aqueous suspension, 448†
- FREIESLEBEN, E. and KJERULF-JENSEN, K.: The effect of thiouracil derivatives on fetuses and infants, 47\*
- FROST, J. W., *see* MCCONNELL, J. S., 812\*
- FURLONG, E., *see* KRICHESKY, B., 448†
- GABRILOVE, J. L.: Chloride excretion during glycosuria in patients with diabetes, 231
- , *see* SOFFER, L. J., 532
- GALLI-MAININI, C.: Pregnancy test using the male toad, 653\*
- , *see* CASTILLO, E. B. DEL, 76
- GASSNER, F. X.: Effect of aqueous testicular extracts on growth and development of spontaneous mammary tumors in the aging bitch, 464†

- GEOGHEGAN, F., *see* SPAIN, A. W., 75  
 GERMEK, O. A., *see* KIMBLE, M. S., 232  
 GLASS, S. J., *see* KRICHESKY, B., 448†  
 GOLDBERG, M. B. and MAXWELL, A. F.: Bilateral arrhenoblastoma without masculinization, adenoma testiculare of Pick, 456†  
 —; MAXWELL, A. F. and SMITH, P. M.: Three unusual endocrinopathies with associated ovarian pathology: I. Ovarian agenesis. II. Precocious puberty. III. Virilism, 11\*  
 —, *see* LISSER, H., 665\*  
 GOLDEN, H., *see* HORVATH, S. M., 73  
 GOLDNER, M. G.: Pheochromocytoma with diabetes, 716\*  
 GOLDSTEIN, N. P.; JACOBSON, M.; TELFORD, I. R., and ROE, J. F.: Studies of pancreatic function. III. The effect of ligation of the pancreatic ducts upon the amylase and lipase content of the blood, 231  
 GOLDSTINE, M. T.: Arrhenoblastoma of the ovary; report of two cases, 606  
 GORDON, A. S.; KADOW, P. C.; FINKELSTEIN, G., and CHARIPPER, H. A.: The thyroid and blood regeneration in the rat, 306  
 GRANT, R. S., *see* SPANKUS, W. H., 586\*  
 GRASSO, R. and ROBERTIS, E. DE: Studies on thyroid stimulating hormone using the cytological method. I. Circulating thyroid stimulating hormone in rats treated with thiourea, 378  
 GREENBLATT, R. B., *see* KUPPERMAN, H. S., 463†  
 —, *see* NIEBURGS, H. E., 450†  
 GREENHILL, M. H.: A psychosomatic evaluation of the psychiatric and endocrinological factors in the menopause, 226  
 GREENWALD, I.: Letter to Editor re "Is endemic goiter due to lack of iodine?," 60\*  
 GROLLMAN, A., *see* HUFFMAN, M. N., 453†  
 GUTMANN, D.: Medullary suprarenal chromaffinoma producing malignant hypertension, 533  
 HAIN, A. M.: The constitutional type of precocious puberty, 171\*  
 HAINES, S. F., *see* DOBYNS, B. M., 306  
 —, *see* SIGLIN, I. S., 433\*  
 HALEY, A. E., *see* SWEENEY, J. S., 659\*  
 HAMBLIN, E. C.: Some contributions of endocrinology to obstetrics and gynecology, 607  
 HAMILTON, J. B.: A secondary sexual character that develops in an organ common to both sexes but normally only in men: with a discussion of the relation of this character to endocrine stimulation, 465†  
 HANDELMAN, N. I., *see* LIKINS, C. H., 780  
 HANSON-PRUSS, O. C.: Thiouracil in treatment of leukemia, 780  
 HARDING, F. E.: The oral use of hexestrol estrogen deficiency, 607  
 HARTMAN, F. A.; BROWNELL, K. A., and THATCHER, J. S.: A new hormone of the adrenal cortex, 461†  
 HAUCK, H. M.: Plasma levels and urinary excretion of ascorbic acid in women during the menstrual cycle, 607  
 HAY, E. C.: The adrenotrophic, renotropic and cardiotropic activities of lyophilized anterior pituitary in thyroidectomized rats, 303  
 HELLER, C. G., *see* JUNGCK, E. C., 1\*  
 HENRY, J. S., *see* BROWNE, J. S. L., 446†  
 HERTZ, A. and ROBERTS, A.: Radioactive iodine in the study of thyroid physiology. VII. The use of radioactive iodine therapy in Graves' disease, 378  
 HERZSTEIN, J. and DOLGER, H.: The fetal mortality in women during the premenstrual period, 727  
 HEWLETT, J. S., *see* McCULLAGH, E. P., 63  
 HIGGINS, G. M. and JONESON, O. R.: Effects of graded doses of thyroxine on experimental goiters, induced by promizone, 378  
 HILLS, A. G.; FORSHAM, P. H., and FINCH, C. A.: Changes in circulating leukocytes induced by pituitary adrenocorticotrophic hormone (ACTH) in man, 458†  
 HORVATH, S. M.; GOLDEN, H., and WAGENET, J.: Some observations on men sitting quietly in extreme cold, 73  
 HOTCHKISS, R. S., *see* MACLEOD, J., 605  
 HOWARD, J. E. and WALSH, F. B.: Conjunctival and corneal lesions in hypercalcemia, 464†  
 —, *see* CALKINS, E., 475\*  
 —, *see* WALSH, F. B., 644\*  
 HUFFMAN, M. N. and GROLLMAN, A.: The metabolic pathway of estradiol production in the organism, 453†  
 HURWITZ, D., *see* SMITH, O. W., 609  
 INGLE, D. J.: The effect of diethylstilbestrol upon alloxan diabetes as related to food intake in the rat, 449†  
 INGRAHAM, F. D. and SCOTT, H. W., JR.: Craniopharyngiomas in children, 376  
 JACKSON, A. S.: Thiouracil will not replace thyroidectomy, 77  
 JACKSON, R. L. and KELLY, H. G.: Growth of children with diabetes mellitus in relation to level of control of the disease, 727

- JACOBS, M., *see* SOFFER, L. J., 532  
 JACOBSON, M., *see* GOLDSTEIN, N. P., 231  
 JAFFE, H.; SOLOMON, B., and WILLIAMS, R. H.: Color reactions of the steroids, 454†  
 —, *see* DAUGHADAY, W. H., 454†  
 JANES, R. G.: The permanency of alloxan diabetes and the structure of the pancreatic islets following certain experimental procedures, 469†  
 JAUDON, J. C.: Addison's disease in children, 383  
 —: Hypofunction of the adrenals in early life, 533  
 JENNINGS, A. F., *see* BROWN, W. E., 450†  
 JEPSON, E. M., *see* THOMPSON, W. O., 467†  
 JONES, C. E. S., *see* FOOTE, E. C., 606  
 JONES, G. S. and EVERETT, H. S.: Arrhenoblastoma of the ovary, with a report of two cases, 607  
 JONES, H. W., *see* LERMAN, J., 77  
 JONESON, O. R., *see* HIGGINS, G. M., 378  
 JUNGCK, E. C.; MADDOCK, W. O. and HELLER, C. G.: Gonadotropic hormone: comparison of ultrafiltration and alcohol-precipitation methods of recovery from urine, 1\*  
 KADOW, P. C., *see* GORDON, A. S., 306  
 KARNAKY, K. J.: Hydrogen ion concentration of the senile vaginal mucosa before and after estrogenic therapy, 226  
 KEATING, F. R., JR., *see* ROGERS, H. M., 383  
 KELLY, H. G., *see* JACKSON, R. L., 727  
 KEPLER, E. J. and MASON, H. L.: Relation of urinary steroids to the diagnosis of adrenal cortical tumors and adrenal cortical hyperplasia: quantitative and isolation studies, 543\*  
 KESMODEL, K. F.: Carcinoma of the breast, 74  
 KEYNES, G., *see* LINNELL, J. W., 77  
 KIBLER, R., *see* KOEPF, G. F., 462†  
 KIMBALL, O. P.: Letter to Editor re "Is endemic goiter due to lack of iodine?" 58\*  
 KIMBLE, M. S.; GERNIEK, O. A., and SEVINGHAUS, E. L.: Vitamin A and carotene metabolism in the diabetic as reflected by blood levels, 232  
 KINSELL, L. W.: Spermatogenesis in a "panhypopituitary" eunuchoid, as the result of testosterone therapy, 781\*  
 KISSIN, M. and BAKST, H.: Co-existing myxedema and hyperparathyroidism: case report, 152\*  
 KJERULF-JENSEN, K., *see* FREIESLEBEN, E., 47\*  
 KLINGER, H. M., *see* FOSS, H. L., 377  
 KOEPF, G. F. and KIBLER, R.: Pellet therapy with desoxycorticosterone acetate in adrenal cortical insufficiency, 462†  
 KOETS, P., *see* DAVISON, R. A., 201\*  
 KRICHESKY, B.; GLASS, S. J.; FURLONG, E., and FEINER, M.: Liver and gonadal changes following the administration of carbon tetrachloride to male rats and female guinea pigs, 448†  
 KULASAVAGE, R. J., *see* LIMARZI, L. R., 225  
 KUPPERMAN, H. S. and GREENBLATT, R. B.: Relationship of sex steroids to the adrenal glands of hamsters and rats, 463†  
 —; MEYER, R. K., and FINERTY, J. C.: Gonadal stimulation following the administration of antigonadotropic serum, 447†  
 —, *see* NIEBURGS, H. E., 450†  
 KUZELL, W. C., *see* DAVISON, R. A., 201\*  
 LAHEY, F. H.: Surgery of the thyroid gland, 379  
 LANG, E. H., *see* PAGE, R. C., 233  
 LANGNER, P. H.; ROMANSKY, M. J., and ROBIN, E. D.: The fallacy of the Exton-Rose glucose tolerance test, 232  
 LAQUEUR, ERNST: Obituary, 603  
 LAZAROW, A. and PALAY, S. L.: The production and course of alloxan diabetes in the rat, 233  
 LEATHEM, J. H. and RAKOFF, A. E.: Equine pituitary gonadotropin and antihormone formation, 466†  
 LEBLOND, C. P., *see* PUPPEL, I. D., 380  
 LEEK, J. H., *see* BAKER, B. L., 383  
 LERMAN, J.; JONES, H. W., and CALKINS, E.: Studies on two sporadic cretinous brothers with goiter, together with some remarks on the relation of hyperplasia to neoplasia, 77  
 LEVINE, E. B. and SELLERS, A. L.: Testosterone in angina pectoris 74  
 LEVINE, R., *see* SCHNEEBERG, N. G., 624\*  
 LEWIS, L. A. and McCULLAGH, E. P.: Plasma protein pattern (Tiselius electrophoretic technique) in Cushing's syndrome, 559\*  
 — and PAGE, I. H.: Further studies on the protective power of adrenal preparations against bacterial toxins, 460†  
 — and PAGE, I. H.: Method of assaying steroids and adrenal extracts for protective action against toxigenic material (typhoid vaccine), 69  
 LI, C. H.: Recipient of Ciba Award of the Association, 223  
 —, *see* MASON, H. L., 458†  
 LIKINS, C. H., JR.; SCOTT, E. P., and HANDELMAN, N. L.: Laurence-Moon-Biedl syndrome, 780  
 LILLIE, R. J., *see* BRIGGS, G. M., 76  
 LIMARZI, L. R.; PIRANI, C. L., and KULA-



- SAVAGE, R. J.: The effect of thiouracil on leukemia with a clinicopathologic report of a case of chronic myeloid leukemia that developed an extreme neutropenic leukopenia, 225
- LINNELL, J. W.; KEYNES, G., and PIERCY, J. E.: Some vulgar errors in regard to goiter, 77
- LIPETZ, B., *see* FELDMAN, F., 376
- LISSE, H.; CURTIS, L. E.; ESCAMILA, R. F., and GOLDBERG, M. B.: The syndrome of congenitally aplastic ovaries with sexual infantilism, high urinary gonadotropins, short stature and other congenital abnormalities. Tabular presentation of twenty-five previously unpublished cases, 665\*
- LIVISON, A., *see* DORFF, G. B., 807\*
- LUBIN, S.: The routine use of stilbesterol for engorgement and lactation in nonnursing mothers, 607
- LUCHETTI, S. E., *see* CASTILLO, E. B. DEL, 76
- LUKENS, F. D. W.: Pituitary-diabetes, 304
- LUND, C. J., *see* WELLS, L. J., 192\*
- LYON, R.: Pregnandiol excretion at the onset of labor, 608
- MACK, H. C. and PARKS, A. E.: The pregnandiol precipitation test—clinical application of a rapid method for the diagnosis of pregnancy, 351\*
- MACLEOD, J. and HOTCHKISS, R. S.: Semen analysis in 1500 cases of sterile marriages, 605
- MADDOCK, W. O., *see* JUNGCK, E. C., 1\*
- MARK, J. S., *see* SHELTON, E. K., 465†, 708\*
- MARTIN, H. E., *see* EDMONDSON, H. A., 471
- MARVIN, H. N., *see* SCHWANDER, H., 423\*
- MASON, H. L.; POWER, M. H.; RYNEARSON, E. H.; CIARAMELLI, L. C.; LI, C. H., and EVANS, H. M.: Results of administration of anterior pituitary adrenocorticotrophic hormone to a human subject, 458†
- , *see* KEPLER, E. J., 543\*
- MASSON, G.: The spermatogenic activity of  $\Delta^5$ -pregnenolone and of its esters, 227
- MATHEWS, K. P., *see* CONN, J. W., 69
- MAXWELL, A. F., *see* GOLDBERG, M. B., 11\*, 456†
- MARTHUR, J. W., *see* RAWSON, R. W., 235\*
- MCCLENDON, J. F. and FOSTER, W. C.: Goiter on an iodine-free diet grown by hydroponics and excluding any goiter noxa, 468†, 714\*
- MCCONNELL, J. S.; FROST, J. W.; WILBUR, R. W., and ROSE, E.: The treatment of thyrotoxicosis with aminothiazole. Results in twenty-three cases, 812\*
- MCCORMACK, G.: A comparison of the color chemical test with the Friedman modification of the Aschheim-Zondek test, 608
- MCCULLAGH, E. P. and HEWLETT, J. S.: Acromegaly associated with amyotrophic lateral sclerosis and acromegaly of the amyotrophic type, 636\*
- , *see* LEWIS, L. A., 559\*
- MCDONALD, D. F. and ODELL, L. D.: Serum glucuronidase activity during normal and toxemic pregnancy, 535\*
- MCGAVACK, T. H., *see* DREKTER, I. J., 451†, 795\*
- , *see* SCHWIMMER, D., 468†
- MEANS, J. H.: Evaluation of the several methods for treating Graves' disease available today, 78
- MEITES, J. and TURNER, C. W.: Effect of thiouracil and estrogen on lactogenic hormone and weight of pituitaries of rats, 531
- MELVIN, J. P., JR., *see* ENGELHARDT, H. T., 230
- MENDEL, E. B.: Chiari-Frommel syndrome: a historical review with case report, 608
- MEYER, R. K., *see* KUPPERMAN, H. S., 447†
- MICHAELS, J. P., *see* NELSON, E. W., 531
- MILLER, H. C.: The effect of diabetic and prediabetic pregnancies on the fetus and newborn infant, 728
- MILLER, R. A.: Pituitary hypothyroidism with impaired renal function, 74
- MINTZ, S. S., *see* TRASOFF, A., 78
- MOEHLIG, R. C.: Addison's disease followed for nine years: case report with autopsy, 134\*
- MORLOCK, C. G., *see* ROGERS, H. M., 383
- MORROW, A. G. and BENUA, R. S.: An evaluation of the Guterman pregnancy test, 608
- MOSCHCOWITZ, E.: Pathogenesis of cirrhosis of the liver occurring in patients with diffuse toxic goiter, 379
- MOSENTHAL, H. O. and ROSEN, A. P.: Insulin regulation in one hundred and twenty-six diabetic children, 205\*
- MURPHY, A. J., *see* FOLDES, F. F., 76
- NELSON, E. W. and MICHAELS, J. P.: Acute postpartum necrosis of the anterior hypophysis, 531
- NEWMAN, E. A. and ROSS, P. H.: Thyrotoxicosis complicated by severe iodism: preparation for surgery with propylthiouracil, 212\*
- NICHOLSON, W. M.: Emotional factors in obesity, 225
- NIEBURGS, H. E.; KUPPERMAN, H. S., and GREENBLATT, R. B.: Studies on the variations of blood gonadotropins and

- vaginal smears during pregnancy in correlation with the fetal sex, 450†
- NOBLE, R. L., *see* TOBY, C. G., 461†
- NORRIS, E. H.: Anatomical evidence of prenatal function of the human parathyroid glands, 383
- ODELL, L. D., *see* McDONALD, D. F., 535\*
- OSHRY, E., *see* SEIDLIN, S. M., 467†
- ØSTERGAARD, E.: Feminizing tumor of the testis—presumably aberrant adrenocortical tumor, 438\*
- PAGE, I. H., *see* CORCORAN, A. C., 801\*
- , *see* LEWIS, L. A., 69, 460†
- PAGE, R. C. and LANG, E. H.: Study of absorption from crystalline insulin pellets and solutions at various sites in rabbits, 233
- PALAY, S. L., *see* LAZAROW, A., 233
- PARKS, A. E., *see* MACK, H. C., 351\*
- PARSON, W., *see* SEGALOFF, A., 446†, 130\*
- PASCHKIS, K. E. and CANTAROW, A.: Hyperophthalmopathic syndrome in thyroid disease, 102\*
- ; CANTAROW, A., and RAKOFF, A. E. Studies in cases of pituitary tumors, 466†
- , *see* RAKOFF, A. E., 448†, 688\*
- PEARSON, P. B., *see* ELLIOTT, J. E., 73
- PEARSON, S., *see* DREKTER, I. J., 451†, 795\*
- PEDERSEN, J.: Virilizing ovarian tumors, 115\*
- PHELPS, D. H.: The factor of previous treatment in experimental menstruation, 449†, 611\*
- PIERCY, J. E., *see* LINNELL, J. W., 77
- PIRANI, C. L., *see* LIMARZI, L. R., 225
- PLANCK, E. H.: A comparison of the effectiveness of radiation therapy and estrogenic substances in the management of hyperthyroidism, 380
- POLLACK, A., *see* SOFFER, L. J., 532
- POMMERENKE, W. T., *see* VIERGIVER, E., 610
- PORCILE, E., *see* BARMAN, J. M., 304
- POTTENGER, F. M., JR.: The oral use of crude adrenal cortex in the stimulation of growth of the face, particularly the condyle of the mandible, 457†
- POWER, M. H., *see* MASON, H. L., 458†
- PREECE, J., *see* CHESLEY, J. C., 605
- PRUNTY, F. T. G., *see* FORSHAM, P. H., 459†
- , *see* THORN, G. W., 459†
- PUPPEL, I. D.; LEBLOND, C. P.; RILEY, E., and CURTIS, G. M.: The clinical significance of the functional behavior of adenomas of the thyroid gland, 380
- ; PASCHKIS, K. E., and CANTAROW, A.: Clinical evaluation of dienestrol, a synthetic estrogen, 448†, 688\*
- , *see* LEATHEM, J. H., 466†
- , *see* PASCHKIS, K. E., 466†
- RAWSON, R. W. and MCARTHUR, J. W.: Radio iodine: its use as a tool in the study of thyroid physiology, 235\*
- REID, D. E.: Treatment of prolonged labor with posterior pituitary extract, 532
- REIFENSTEIN, E. C., JR., *see* FORBES, A. P., 264\*
- REITMAN, F., *see* DAX, E. C., 533
- REVENO, W. S.: Observations on the use of thiouracil, 380
- : Thiouracil effect in diabetes mellitus complicated by hyperthyroidism, 234
- REYNOLDS, F. W., *see* CONNOR, J. F., 229
- REYNOLDS, S. R. M.: Distortion of the spiral artery in the ovary in the presence of corpus hemorrhagicum cysts after administration of gonadotropins to rabbits, 447†
- : The relation of hydrostatic conditions in the uterus to the size and shape of the conceptus during pregnancy: a concept of uterine accommodation, 474
- RICHTER, C. P.: Carbohydrate appetite of normal and hyperthyroid rats as determined by the taste-threshold method, 467†
- RIDDLE, O. and associates: Studies on carbohydrate and fat metabolism. With special reference to the pigeon. (Book review), 661
- RILEY, E., *see* PUPPEL, I. D., 380
- ROBBINS, J., *see* CARTER, A. C., 464†, 753\*
- ROBERTIS, E. DE, *see* GRASSO, R., 378
- ROBERTS, A., *see* HERTZ, A., 378
- ROBERTS, J. B., *see* FELDMAN, F., 376
- ROBERTS, J. G.: Disappearance of secondary sarcomatous deposits in the lungs after stilbesterol therapy, 226
- ROBIN, E. D., *see* LANGNER, P. H., 232
- ROE, J. F., *see* GOLDSTEIN, N. P., 231
- ROGERS, H. M.; KEATING, F. R., JR.; MORLOCK, C. G., and BARKER, W. W.: Primary hypertrophy and hyperplasia of the parathyroid glands associated with duodenal ulcer, 383
- ROGERS, W. F., JR. and WILLIAMS, R. H.: Correlations of biochemical and histological changes in the adrenal cortex in various types of disease, 463†
- ROGERS, W. F., JR., *see* WILLIAMS, R. H., 462†
- ROMANSKY, M. J., *see* LANGNER, P. H., 232
- ROOT, H. F.; STOTZ, E., and CARPENTEN, T. M.: The respiratory quotient and blood pyruvate and lactate responses
- RAKOFF, A. E.: Studies on high dosage progesterone therapy of amenorrhea, 609

- after oral ingestion of glucose and fructose in diabetes mellitus with and without insulin, 472
- ROSE, EDWARD, *see* McCONNELL, J. S., 812\*
- ROSENFELD, S., *see* ZWEIFACH, B. W., 460†
- ROSS, E., *see* SHIPLEY, R. A., 70
- ROSS, P. H., *see* NEWMAN, E. A., 212\*
- ROSSMAN, I. and BARTELMEZ, G. W.: Delayed ovulation, a significant factor in the variability of the menstrual cycle, 609
- RUTHERFORD, R. N.: Preconceptional progestin therapy in habitual abortion, 609
- RYNEARSON, E. H., *see* MASON, H. L., 458†
- SALTER, W. T.: The applications of the Zimmermann and the Kober reactions concomitantly to human urine, 453†
- SALTZMAN, A. H., *see* TALBOT, N. B., 331\*
- SCHERMANN, J., *see* CAPRIGLIONE, L., 303
- SCHNEEBERG, N. G.; WOOLHANDLER, G., and LEVINE, R.: The clinical significance of hyperostosis frontalis interna, 624\*
- SCHNEIDER, C. L.: The active principle of placental toxin: thromboplastin; its inactivator in blood: antithromboplastin, 473
- SCHWANDER, H. and MARVIN, H. N.: Treatment of carcinoma of the human breast with testosterone propionate, 423\*
- SCHWIMMER, D.; VOGEL, M., and MCGAVACK, T. H.: Clinical manifestations in forty cases of myxedema, 468†
- SCOTT, E. P., *see* LIKINS, C. H., JR., 780
- SCOTT, H. W., JR., *see* INGRAHAM, F. D., 376
- SEGALOFF, A.: The effect of diet on the growth and survival of adrenalectomized rats treated with desoxycorticosterone acetate pellets, 70
- and PARSON, W.: Hypogonadotropic eunuchoidism: report of case with failure to respond to chorionic gonadotropic hormone due to antihormones, 130\*
- ; WEED, J. C., and PARSON, W.: Progesterone therapy of uterine fibromyoma, 446†
- , *see* WEED, J. C., 455†, 741\*
- SEIDLIN, S. M.; OSHRY, E., and YALOW, A. A.: Twelve cases of metastatic thyroid carcinoma studied with radioactive iodine, 467†
- SELEY, A. D.; BAUMGOLD, D., and VERNICK, S.: Oral estrogen therapy during menopause, 451†
- SELIGMAN, B. and WEINTROB, M.: (Letter to Editor). Treatment of thiouracil agranulocytosis with streptomycin, 219\*
- SELLERS, A. L., *see* LEVINE, E. B., 74
- SEVRINGHAUS, E. L., *see* KIMBLE, M. S., 232
- SEXTON, D. L.: Thiouracil. Clinical evaluation following two and one-half years' experience, 381
- SHARPEY-SHAFER, E. P.: 2-Thiouracil in the treatment of congestive heart failure, 74
- SHELTON, E. K. and MARK, J. S.: The use of methyl testosterone and testosterone propionate in premature infants, 465†
- ; VARDEN, A. E., and MARK, J. S.: Experimental use of testosterone compounds in premature infants, 708\*
- SHIPLEY, R. A. and DORFMAN, R. I.: The effect of infection and trauma on the excretion of urinary cortin, 70
- ; DORFMAN, R. I.; BUCHWALD, E., and ROSS, E.: The effect of infection and trauma on the excretion of urinary cortin, 70
- , *see* DUMM, R. M., 230
- SHORR, E., *see* ALMY, T. P., 455†
- , *see* CARTER, A., 828\*
- , *see* ZWEIFACH, B. W., 460†
- SIGLIN, I. S.; EATON, L. M.; CAMP, J. D., and HAINES, S. F.: Symmetric cerebral calcification which followed postoperative parathyroid insufficiency: report of a case, 433\*
- SIMKINS, S.: Use of massive doses of vitamin A in the treatment of hyperthyroidism. A preliminary report, 574\*
- SMITH, E. J. R., *see* DAX, E. C., 533
- SMITH, G. V. S., *see* SMITH, O. W., 609
- SMITH, O. W.; SMITH, G. V. S., and HURWITZ, D.: Increased excretion of pregnanediol in pregnancy from diethylstilbesterol with special reference to the prevention of late pregnancy accidents, 609
- SMITH, P. M., *see* GOLDBERG, M. B., 11\*
- SOFFER, L. J.; VOLTERRA, M.; GABRILOVE, J. L.; POLLACK, A., and JACOBS, M.: Effect of iodine and adrenalin on thyrotropin in Graves' disease and in normal and thyroidectomized dogs, 532
- SOLOMON, B., *see* JAFFE, H., 454†
- SPAIN, A. W. and GEOGHEGAN, F.: Diabetes insipidus with postpartum pituitary necrosis. A report of two cases, 75
- SPALDING, J. M. K.: A case of phaeochromocytoma, 534
- SPANKUS, W. H. and GRANT, R. S.: Gynecomastia, 586\*
- SPECK, G.: Pregnancy in cases of pituitary dwarfism, 532
- SPENCE, H. M. and THOMPSON, F. G., JR.: Hormone-producing tumor of adrenal cortex with congenital absence of contralateral adrenal gland, 71
- STAFFIERI, J. J., *see* CASTILLO, E. B. DEL, 76
- STAINSBY, W. J. and COLLINS, J. A., JR.: Diabetes mellitus. An analysis of 250

- cases and a discussion of simplified procedures for regulation, 779
- STEALY, C. L., *see* STIMMEL, B. F., 452†
- STIMMEL, B. F.: The metabolism of single therapeutic doses of the natural estrogens in human subjects, 364\*
- : Some observations on the utilization of a liquid chromatogram technique in the colorimetric estimation of urinary pregnanediol, 457†
- and STEALY, C. L.: Further studies on the metabolism of therapeutic doses of the natural estrogens in human subjects, 452†
- STOTZ, E., *see* ROOT, H. F., 472
- STURGIS, C. C., *see* BEIERWALTES, W. H., 305
- SULMAN, F., *see* ZONDEK, B., 159\*
- SUSSELMAN, S., *see* FELDMAN, F., 376
- SWEENEY, J. S.; HALEY, A. E., and YAKLIN, L.: (Letter to Editor). An observation on menstrual misbehaviors, 659\*
- TALBOT, N. B.; ALBRIGHT, F.; SALTZMAN, A. H.; ZYGMUNTOWICZ, A., and WIXOM, R.: The excretion of 11-oxy corticosteroid-like substances by normal and abnormal subject, 331\*
- TEILUM, G.: Arrhenoblastoma—androblastoma. Homologous ovarian and testicular tumors II, 228
- : Gonocytoma. Homologous ovarian and testicular tumors I, 227
- TELFORD, I. R., *see* GOLDSTEIN, N. P., 231
- THATCHER, J. S., *see* HARTMAN, F. A., 461†
- THELANDER, H. E.: Congenital adrenal-cortical insufficiency associated with macrogenitosomia, 604
- THOMPSON, F. G., JR., *see* SPENCE, H. M., 71
- THOMPSON, K. W.: Letter to Editor re "Is endemic goiter due to lack of iodine?" 62\*
- THOMPSON, P. K., *see* THOMPSON, W. O., 467†
- THOMPSON, W. O.; THOMPSON, P. K., and JEFFSON, E. M.: The effect of hypothyroidism on menstruation, 467†
- THORN, G. W.; PRUNTY, F. T. G., and FORSHAM, P. H.: Metabolic changes following the administration of pituitary adrenocorticotrophic hormone (ACTH) in man, 459†
- , *see* FORSHAM, P. H., 459†
- TOBY, C. G. and NOBLE, R. L.: The role of the adrenal cortex in protein catabolism following trauma, 461†
- TOWERY, B., *see* WILLIAMS, R. H., 462†
- TRABUCCO, A., *see* CASTILLO, E. B. DEL, 493\*
- TRASOFF, A.; WOHL, M. G., and MINTZ, S. S.: Fatal agranulocytosis with autopsy following use of thiouracil in case of thyrotoxicosis, 78
- TURNER, C. W., *see* MEITES, J., 531
- UCKO, H.: Iodine-lack theory and endemic goiter, 820\*
- VANDERLAAN, W. P., *see* ASTWOOD, E. B., 304
- VARDEN, A. E., *see* SHELTON, E. K., 708\*
- VENNING, E. H. and BROWNE, J. S. L.: Effect of testosterone upon the excretion of glycoenic corticoids, 460†, 729\*
- and BROWNE, J. S. L.: Excretion of glycoenic corticoids and of 17-ketosteroids in various endocrine and other disorders, 79\*
- , *see* BROWNE, J. S. L., 446†
- VERNICK, S., *see* SELEY, A. D., 451†
- VIERGIVER, E. and POMMERENKE, W. T.: Cyclic variations in the viscosity of cervical mucus and its correlation with amount of secretion and basal temperatures, 610
- VOGEL, M., *see* SCHWIMMER, D., 468†
- VOLTERRA, M., *see* SOFFER, L. J., 532
- WAGER, J., *see* HORVATH, S. M., 73
- WALSH, F. B. and HOWARD, J. E.: Conjunctival and corneal lesions in hypercalcemia, 644\*
- , *see* HOWARD, J. E. 464†
- WEAVER, J. D.: Estrogenic hormones, often only a psychotherapeutic agent, 228
- WEED, J. C.; SEGALOFF, A.; WIENER, W., and DOUGLAS, J. W.: True hermaphroditism. Endocrine studies in a case of ovotestis, 741\*
- , SEGALOFF, A.; WIENER, W., and DOUGLAS, J. W.: True hermaphroditism: Report of a case with an ovotestis, and endocrine studies, 455†
- , *see* SEGALOFF, A., 446†
- WEINTROB, M., *see* SELIGMAN, B., 219\*
- WEISMAN, A. I. and COATES, C. W.: The diagnosis of hydatidiform mole by gonadotropic hormone assay using the South African frog, *Xenopus laevis*, 289\*
- WELLS, L. J. and LUND, C. J.: Effects of steroid hormones upon the developmental separation of the prepuce from the glans penis, 192\*
- WEST, R., *see* COVOLO, G. C., 325\*
- WETZEL, N. C.: The baby grid. An application of the grid technique to growth and development in infants, 780
- WHITE, P.: Pregnancy complicating diabetes, 471
- WHITELAW, M. J.: Thiouracil in the treatment of hyperthyroidism complicating

- pregnancy and its effects on the fetus, 469†
- : Thiouracil in the treatment of hyperthyroidism complicating pregnancy and its effect on the human fetal thyroid, 767\*
- WIENER, W., *see* WEED, J. C., 455†, 741\*
- WILBUR, R. W., *see* McCONNELL, J. S., 812\*
- WILKINS, L. and FLEISCHMANN, W.: Effects of thyroid on creatine metabolism with a discussion of the mechanism of storage and excretion of creatine bodies, 381
- WILLIAMS, R. H.: Thyroid and adrenal interrelations with special reference to hypotrichosis axillaris in thyrotoxicosis, 52\*
- ; DAUGHADAY, W. H.; ROGERS, W. F., Jr.; ASPER, S. P., Jr., and TOWERY, B.: Studies on obesity, 462†
- , *see* DAUGHADAY, W. H., 454†
- , *see* JAFFE, H., 454†
- , *see* ROGERS, W. F., Jr., 463†
- WILLIAMSON, M. B.: Concentration and properties of the adrenocorticotrophic substance in female human urine, 71
- WINSON, S. G.: An analysis of 257 cases of sterility, 610
- WINTHER, N.: Menorrhoeal problems in college women, 610
- WIXOM, R., *see* TALBOT, N. B., 331\*
- WOHL, M. G., *see* TRASOFF, A., 78
- WOLMAN, I. J.: Basal metabolism in childhood: current progress, 382
- : Melituria in healthy American men with special reference to transitory glycosuria, 473
- WOOD, M. N.: Chronic peptic ulcer in 94 diabetics, 779
- WOOLHANDLER, G., *see* SCHNEEBERG, N. G., 624\*
- YAKLIN, L., *see* SWEENEY, J. S., 659\*
- YALOW, A. A., *see* SEIDLIN, S. M., 467†
- YOUNG, F. G.: Growth and diabetes in normal animals treated with pituitary (anterior lobe) diabetogenic extract, 75
- ZONDEK, B. and BLACK, R.: Estrone clearance test in infectious hepatitis, 519\*
- and SULMAN, F.: The hyperemia AZT and the evaluation of the hyperemia rat unit of chorionic gonadotropin, 159\*
- ZWEIFACH, B. W.; SHORR, E.; BAEZ, S., and ROSENFELD, S.: Hepato-renal factors in circulatory homeostasis: XVIII, Relation of adrenals to formation of a renal vaso-excitor principle, 460†
- ZYGMUNTOWICZ, A., *see* TALBOT, N. B., 331\*

# SUBJECT INDEX

\* Original article.

† Abstract of paper presented at the Twenty-ninth Annual Meeting of The Association for the Study of Internal Secretions.

Other items are abstracts of articles which appeared in current medical journals.

**ABNORMALITIES:** *see* Congenital Anomalies; and under various endocrine glands

**ABORTION:** *see* Pregnancy

**ABSORPTION:** *see also* Pellet implantation; and under various preparations

— effect of methods of administration on, 452†

— of diethylstilbestrol dipalmitate, comparison of aqueous and oil preparations, 448†

— of insulin in rabbits, administered by different routes, 233

**ABSTRACTS:** *see* Association

**ACROMEGALY:** *see* Pituitary, disorders

**ADDISON'S DISEASE:** *see also* Adrenals disorders, preparations, steroids, etc.

— absence of adrenals in, 134\*

— cholelithiasis associated with, 134\*

— familial, 134\*

— followed nine years; autopsy; diffuse fibrosis of anterior pituitary, etc., 134\*

— in children, 383

— in the Negro, 69

**BLOOD IN,** leukopenia, severe, 134\*

— leukocytes, circulating; effect of adrenocorticotrophic hormone on, 458†

— red blood cells, arginase activity of; effect of 11-dehydrocorticosterone acetate on, 325\*

**SKIN IN,** leukoderma or vitiligo, 134\*

— in Negro, 69

— pigmentation, intense, 134\*

**THERAPY,** adrenal cortex extract; vitamin

C; salt; pellet implantation: intramuscular and sublingual administration of desoxycorticosterone acetate; in patient followed 9 years, 134\*

— adrenocorticotrophic hormone; effect on circulating leukocytes in, 458†

— 11-dehydrocorticosterone acetate; effect on arginase activity of red blood cells, 325\*

— desoxycorticosterone acetate in treatment of adrenal cortical insufficiency; by implantation of pellets, 70, 134\*, 331\*, 462†  
by injection, 331\*, 533

**URINE IN,** adrenocortical hormones; chemical assay of, 454†

— glycogenic corticoids, 79\*

— gonadotropins, 1\*

— 17-ketosteroids, 79\*, 331\*

— 11-oxycorticosteroids, 331\*

## ADRENALS

**ABNORMALITIES:** *see also* Congenital Anomalies

— *aberrant adrenal tissue*; in bilateral arrhenoblastoma without masculinization, 456†

— in location of rudimentary ovary, 385\*

— in ovarian tumors, 115\*, 605

— in testes, 604

— in testicular tumor, 438\*

— *absence*, congenital, of one adrenal; tumor in other, 71

— *absence of*, in case of Addison's disease, 134\*

## DISORDERS

— *adrenal cortical insufficiency*, 462†

— in early life, 533

— with diabetes mellitus and hypopituitarism (post-thyroidectomy), 376

— with macrogenitosomia, 604

— *familial*: *see* Familial

— *hyperadrenocorticism*, with Cushing's syndrome, 331\*, 787\*

— with virilism, 331\*

— *of cortex*: *see also* Addison's disease; Adrenals, tumors; Cushing's syndrome; Puberty, precocious; Virilism

— *of medulla*: *see* Pheochromocytoma; Tumors

— *therapy*: *see* Adrenals, preparations

— *urine*: *see* Adrenals, steroids

## EXCISION

— desoxycorticosterone acetate pellets and diet in treatment of adrenalectomized rats, 70

— unilateral, in mental disorder associated with virilism and with pseudohermaphroditism, 533

## HORMONES: *see also* Adrenals, steroids

— adrenocortical hormones in endocrine and non-endocrine diseases, chemical assay of, in urine, 454†

## ADRENALS (cont.)

## HORMONES (cont.)

- new hormone of adrenal cortex, the "fat factor," 461†

## PHYSIOLOGY

- adrenal-gonad-pituitary relationship in hamsters and rats, 463†
- *adrenocorticotrophic hormone of the pituitary*: see also Pituitary
  - changes in circulating leukocytes induced by, in normal subjects and in Addison's disease, 458†
  - chemical and cytochemical studies of adrenal cortex of rat, following administration of, 457†
  - determination of uric acid-creatinine ratio in urine, following administration of, 459†
  - in female human urine: effect on ascorbic acid level in adrenal, 71
  - in thyroidectomized rats, 303
  - metabolism of electrolytes, nitrogenous principles, steroids, etc. following administration to human subjects, 458†, 459†
- correlation of adrenal histology with 17-ketosteroid excretion in various conditions associated with mental retardation 503\*
- correlations of biochemical and histological changes in various types of disease, 463†
- *function*: see also Steroids
  - androgenic, indicated by axillary hair, 52\*
  - measured by: 17-ketosteroid excretion in urine, 79\*, 264\*, 503\*  
lipoid deposition in adrenal cortex, 463†  
11-oxycorticosteroids, 331\*
  - simple test for, by use of uric acid-creatinine ratio in urine following administration of ACTH, 459†
  - types of, differentiation by use of urinary glycogenic corticosteroids and 17-ketosteroids, 79\*
- relation of adrenals to formation of a renal vaso-excitator principle in circulatory hemostasis, 460†
- role of adrenal cortex in protein catabolism following trauma, 461†
- size of adrenals in hamsters and rats, relationship of sex steroids to, 463†
- thyroid interrelations, 52\*

## PREPARATIONS

- adrenalin: effect on serum thyrotropin in Graves' disease, 532

## ADRENALS (cont.)

## PREPARATIONS (cont.)

- adrenal cortex, crude; oral use of in stimulation of growth of face, particularly the condyle of the mandible, 457†
- adrenal cortex extract in treatment of Addison's disease, 134\*
- adrenal extracts and steroids; assay for protective action against toxins, 69
- adrenal preparations, protective power of, against bacterial toxins, 460†
- adreno-cortical preparations in treatment of adrenal cortical insufficiency associated with macrogenitosomia, 604
- 11-dehydrocorticosterone acetate, effect on arginase activity of red blood cells, in Addison's disease and in Simmonds' disease, 325\*
- *desoxycorticosterone acetate*
  - effect on excretion of 11-oxycorticosteroids in adrenal virilism, 331\*
  - in treatment of adrenal cortical insufficiency, 134\*, 533  
by pellet implantation, 70, 134\*, 462†  
by sublingual administration; swelling of salivary glands and salivation due to, 134\*
- epinephrine, in diagnosis and post-operative treatment of pheochromocytoma, 475\*

## STEROIDS

- *excretion in urine*
  - adrenocortical hormones in endocrine and non-endocrine diseases, chemical assay of, 454†
  - "corticosterone" excretion in obesity, 462†
  - cortin, effect of infection and trauma on, 70, 70
  - cortin-like substances, effect of ACTH on, 458†
  - glycogenic corticoids, effect of testosterone on, 460†, 729\*  
in Addison's disease, 79\*
  - 17-ketosteroids
    - effect of ACTH on, 458†, 459†
    - effect of testosterone on, 729\*
    - in Addison's disease, 79\*, 331\*
    - in hyperadrenocorticism with Cushing's syndrome, 331\*
    - in pheochromocytoma with diabetes, 716\*
- 11-oxycorticosteroids, in Addison's disease, 331\*

## ADRENALS (cont.)

## STEROIDS (cont.)

in hyperadrenocorticism with Cushing's syndrome; effect of removal of tumor of adrenal cortex on, 331\*

in hyperadrenocorticism with virilism, 331\*

## — steroids

effect of unilateral adrenalectomy in mental disorder with virilism, ism, etc., 533

relation of, to adrenal cortical hyperplasia and tumors, 543\*

TUMORS: *see also* Adrenals, abnormalities

— absence, congenital, of one adrenal; tumor in other, 71

— adrenal-like tumor of ovary, 605

— carcinoma of adrenal cortex in unusual case of virilism following remission of Cushing's syndrome, 787\*

— differential diagnosis of tumor from hyperplasia, 543\*

— effect of removal of adrenal cortex tumor upon excretion of 11-oxy-corticosteroids and 17-ketosteroids in Cushing's syndrome, 331\*

— increased excretion of dehydroisoandrosterone in, 543\*

— pheochromocytoma: *see* Pheochromocytoma

— relation of urinary steroids to, 543\*

AGENESIS, ovarian: *see* Ovary, syndrome

AGRANULOCYTOSIS: *see* Bloods, cells; Thiouracil

ALLERGY, freedom from local allergic reactions following administration of diethylstilbestrol dipalmitate in aqueous medium, 448†

ALLOXAN: *see* Diabetes mellitus, experimental

AMENORRHEA: *see* Menstruation

AMERICAN ASSOCIATION FOR THE STUDY OF GOITER: *see* Association News

AMERICAN MEDICAL ASSOCIATION: *see* Association News

AMINOTHIAZOLE, in treatment of thyrotoxicosis, 812\*

AMYLASE in blood; effect of ligation of pancreatic ducts on, in cats, 231

ANDROBLASTOMA: *see* Testis

ANDROGENS: *see also* Gonad; Steroids; Testis; Urine

— androgenic function of adrenal indicated by amount of axillary hair, 52\*

— deficiency in male; evaluation of urethral smear test for, 186\*

## ANDROGENS (cont.)

## EFFECTS

— of methyl testosterone

— and testosterone propionate on excretion of creatine and creatinine, 381

— and testosterone propionate on excretion of glycogenic corticoids and 17-ketosteroids in normal subjects, diabetes mellitus, Cushing's syndrome, and lupus erythematosus disseminatus, 729\*

— on arginase activity of red blood cells, 325\*

— on urethral smear in male hypogonadism, 186\*

— of testicular extract, aqueous, on growth and development of mammary tumors in dogs, 464†

— of testosterone, on blood regeneration in thyroidectomized rats, 306

— on excretion of glycogenic corticoids, 460†

— on excretion of 11-oxy-corticosteroids in Cushing's syndrome and in adrenal cortical virilism, 331\*

— on plasma protein pattern in Cushing's syndrome, 559\*

— on spermatogenesis in the rat, 227

of testosterone propionate: *see also* androgens, effects of methyl testosterone, and therapy

— cause of virilism in women treated for carcinoma of breast, 423\*

— on prostate and seminal vesicles, (topical application), 192\*

— on serum calcium, 74

## IN URINE

— androgen-estrogen relationships in human organism, measured by Zimmemann and Kober reactions applied concomitantly to urine, 453†

— androsterone, dehydroisoandrosterone and testosterone; quantitative recovery from urine, 795\*

— dehydroisoandrosterone: increased excretion of, in case of adrenal cortical tumor, 543\*

— effect of removal of virilizing ovarian tumor, on excretion of, 115\*

— in diagnosis of adrenal cortical tumors and hyperplasia; including Cushing's syndrome, precocious puberty and hermaphroditism, 543\*

— in feminizing testicular tumor, 438\*



## ANDROGENS (cont.)

## IN URINE (cont.)

- in human male; excretion of 17-ketosteroids, 452†
- in various conditions associated with mental retardation; including mongolism, cretinism and myotonia dystrophica, 503\*

## THERAPY

- *methyl testosterone*, sublingual application, in treatment of male hypogonadism, 293\*
- therapy in premature infants, 465†, 708\*
- *testosterone*, in treatment of hypogonadotropic eunuchoidism, 130\*
- in treatment of male climacteric, 74
- in treatment of polyostotic fibrous dysplasia: improvement of vision, 455†
- lack of effect in angina pectoris, 74
- pellet implantation: in treatment of male hypogonadism, 293\*
- pellet implantation: in treatment of "pan-hypopituitary" eunuchoidism, resulting in spermatogenesis, 781\*
- *testosterone propionate*, in treatment of acromegaly, 636\*
- in treatment of carcinoma of the female human breast, 74, 423\*
- therapy, in premature infants, 465†, 708\*
- topical application, effect of, on developmental separation of prepuce from glans penis, 192\*

ANEMIA: *see* Blood

ANGINA PECTORIS: *see* Heart

ANNOUNCEMENTS *see* Association

ANNUAL MEETINGS: *see* Association, announcements

ANOMALIES: *see* Congenital anomalies; and abnormalities under various endocrine glands

ANOREXIA NERVOSA, glycogenic corticoid excretion in, 79\*

— 17-ketosteroid excretion in, 79\*

— urinary gonadotropins in, 1\*

ANTI-HORMONES, against pituitary equine gonadotropin, 466†

antigonadotropic, cause of failure to respond to chorionic gonadotropin, in eunuchoidism, 130\*

— determination of, 130\*

antigonadotropic serum, basophilic changes in pituitary following administration to rats, 447†

## ANTI-HORMONES (cont.)

— gonadal stimulation following administration to rats, 447†

ANTIMONY-TRICHLORIDE REACTION in determining chromogenicity of steroids, 454†

ANTITHROMBOPLASTIN, 473

ARGINASE, activity of, in red blood cells; effect of methyl testosterone and of 11-DHCA, 325\*

ARRHIENOBLASTOMA: *see* Ovary, tumors

ARTERIES: *see* Vascular system: Hypertension

ARTHRITIS, ankylosing spondylarthritis (Marie-Strümpell type) and rheumatoid arthritis; 17-ketosteroid excretion in, in relation to sex distribution, 201\*

— arthralgia and urticaria during aminothiazole therapy in thyrotoxicosis, 812\*

ARTIFICIAL INSEMINATION, studies, showing optimal time of conception, 606

ASCHHEIM-ZONDEK TEST: *see* Gonadotropins; Pregnancy, diagnosis; Tests

ASCORBIC ACID: *see* Vitamins

ASSOCIATION FOR THE STUDY OF INTERNAL SECRETIONS

ABSTRACTS of current endocrine literature, 69, 225, 303, 376, 471, 531, 604, 727, 779

— of papers on program, 29th Annual Meeting of the Association, June 6-7, 1947, 446-470†

— reviews, book, 661

ANNOUNCEMENTS OF THE ASSOCIATION

— *annual meetings*

— twenty-ninth, 68, 165, 222, 446

— thirtieth, 530, 602, 662, 724, 774, 831

— *awards*

— Ayerst, McKenna and Harrison fellowship, 663, 725, 775, 832

— Ciba, 663, 725, 775, 832

— E. R. Squibb and Sons, 663, 725, 775, 832

— recipients of, 223

— *postgraduate course in endocrinology*, 777, 834

LETTERS TO THE EDITOR, 58\*, 60\*, 62\*, 219\*, 659\*, 828\*, 830\*

## NEWS

— American Association for the Study of Goiter, Van Meter Prize Award, 603, 664, 726, 778, 833

— American Medical Association, Council on Pharmacy and Chemistry, grants for research, 170

— Cancer Research Congress, Fourth International, 66

— Laurentian Hormone Conference, program, 374

ASSOCIATION FOR THE STUDY OF  
INTERNAL SECRETIONS (cont.)

## NEWS (cont.)

- Obituary, E. L. Laqueur, 603
- Schering award competition, 66
- Schering endocrine research funds, 65

NOTICES: *see* Announcements and news

POSTGRADUATE COURSE in Endocrinology, 777, 834

## PROGRAMS

- of *twenty-ninth annual meeting*, 166-169
- abstracts of papers read, 446-470†

AUTOTRANSPLANTATION of thyroid, role of nervous system in, 75

AWARDS: *see* Association, awards and newsAYERST, McKENNA AND HARRISON FELLOWSHIP: *see* Association, awardsAZOOSPERMIA: *see* Sterility; Testis, spermatozoa

## BASAL METABOLISM, in childhood, 382

- method for assuring accuracy, 304

## BLOOD

- *amylase* and *lipidase* content, effect of ligation of pancreatic ducts on, in cats, 231
- *anemia* induced by hypophysectomy in rats; effects of iron, copper and thyroxine, 376
- *antigonadotropic hormone*, determination of, in eunuchoidism, 130\*
- *arginase*, activity of, in red cells: effect of methyl testosterone and 11-DHCA on, 325\*

CALCIUM determination of, by direct photoelectric method, 73

- hypercalcemia with conjunctival and corneal lesions in vitamin D poisoning, hyperparathyroidism, and sarcoid, 464†, 644\*
- in myxedema with hyperparathyroidism, 152\*
- *carotene* and *vitamin A* metabolism in the diabetic, as reflected by blood levels, 232

## CELLS

- *agranulocytosis*, fatal, from thiouracil in thyrotoxicosis, 78
- from thiouracil in treatment of malignant exophthalmos, 102\*
- from thiouracil; streptomycin therapy in, 219\*
- from thiouracil therapy in toxic goiter, 305

## BLOOD (cont.)

## CELLS (cont.)

- prevention of, during administration of thiouracil, 305
- granulopenia during thiouracil therapy, 566\*
- leukocytes, changes in, induced by pituitary adrenocorticotrophic hormone in normal subjects and in Addison's disease, 458†

## CHOLESTEROL

- cholesterol esters and phospholipid phosphorus in thyroid disease, 76
- cholesterol, in hyperthyroidism, effect of treatment with vitamin A, 574\*
- *estrone*, in acromegaly, cirrhosis of the liver, pregnancy, and pregnancy with infectious hepatitis, 519\*
- *glucuronidase* activity of serum during normal and toxemic pregnancy; effect of stilbestrol on, in normal puerperium, 535\*
- *gonadotropins* in pregnancy; correlation with sex of fetus, 450†
- *hemochromatosis* without pigmentation or diabetes, 72

IODINE, in eunuchoidism with hyperthyroidism, 566\*

- in obesity, 462†

LEUKEMIA, effect of thiouracil on, 225

- thiouracil therapy in, 780

PHOSPHORUS and phosphatase, (alkaline) in myxedema with hyperparathyroidism, 152\*

- phosphorus, phospholipid, in thyroid disease, 76

## PROTEIN

- protein content and specific gravity of plasma in hyperthyroidism, 75
- protein pattern of plasma in Cushing's syndrome; effect of bilateral hemiadenectomy, of testosterone, and of x-ray irradiation of pituitary on, 559\*
- protein pattern of plasma in disorders of adrenal and pituitary, 559\*
- *pyruvate* and *lactate*, in diabetes mellitus, following oral ingestion of glucose and fructose, with and without insulin, 472
- *regeneration*; effect of castration, cobalt, testosterone, thiouracil, thyroxine, etc. on, in rat, 306
- *sedimentation rate*; effect of adrenalectomy on, in combined adrenal and mental disease, 533

## BLOOD (cont.)

- SUGAR: *see also* Carbohydrate metabolism;  
 Diabetes mellitus  
 —hypoglycemia, rapid test for, 72  
 —hypoglycemia, severe, in Addison's disease, 134\*

BODY, growth and weight: *see* Growth; Weight

## BONES, age studies in syndrome of ovarian aplasia with sexual infantilism, high urinary gonadotropins and short stature, 665\*

- alterations in, in "rudimentary ovary" syndrome, 385\*  
 —effect of androgen therapy on osteolytic metastases from carcinoma of human breast, 423\*  
 —hyperostosis cranialis interna in case of virilizing ovarian tumor, 115\*  
 —hyperostosis frontalis interna syndrome not a clinical entity, 624\*  
 —mandible; stimulation of growth by oral use of crude adrenal cortex extract, 457†  
 —osteoporosis and Paget's disease; effect of stilbestrol and progesterone on excretion of 11-oxycorticosteroids in, 331\*  
 —perosis from thiouracil, in chicks, 76  
 —polyostotic fibrous dysplasia; a defense of the entity, 307\*  
 —polyostotic fibrous dysplasia; regression with testosterone therapy; improvement of vision, 455†  
 —relationship of parathyroid glands to action of estrogen on, 383

## BOOK REVIEW, Studies on Carbohydrate and Fat Metabolism, With Especial Reference to the Pigeon. Riddle, Oscar and associates, 661

BRAIN: *see also* Congenital Anomalies; Psychic factors; Psychosis

- cerebral calcification, symmetric, following postoperative parathyroid insufficiency, 433\*  
 —17-ketosteroid excretion in various conditions of defective mental development (oligophrenia); correlation with autopsy findings, 503\*  
 —mental disorders associated with adrenal virilism; effect of adrenalectomy in, 533

BREAST: *see also* Ovary; Tumors

- CARCINOMA, testosterone propionate therapy; cause of hirsutism, 423\*  
 —effect on serum calcium, 74  
 —effect on serum calcium, inorganic phosphorus, and acid and alkaline phosphatase, 423\*

## GYNECOMASTIA in bilateral arrhenoblastoma without masculinization: high urinary gonadotropin titer, 456†

## BREAST (cont.)

## GYNECOMASTIA (cont.)

- in thyrotoxicosis; relation of axillary hair to, 52\*  
 —male, as a result of testicular tumor; effect of removal of tumor, 438\*  
 differential diagnosis, etiology, incidence, pathology, treatment, 586\*  
 following administration of aqueous testicular extract, in dog, 464†  
 in association with androblastoma, 228  
 in eunuchoid males, 566\*, 586\*  
 hormone excretion in, 586\*  
 oligospermia associated with, 586\*

## LACTATION: inhibition of, by dieneestrol, a synthetic estrogen, 448†, 688\*

- use of stilbestrol in prevention of engorgement and lactation in non-nursing mothers, 607

## MASTITIS, chronic cystic, dieneestrol therapy in, 688\*

## TUMORS, effect of aqueous testicular extracts on mammary tumors in dog, 464†

## BURNS, excretion of 17-ketosteroids and 11-oxycorticosteroids in, 331\*

## CALCIUM, calcification, symmetric cerebral, following postoperative parathyroid insufficiency, 433\*

- determination of, in serum, by direct photoelectric method, 73  
 —hypercalcemia, with conjunctival and corneal lesions, in vitamin D poisoning, in hyperparathyroidism, and in sarcoid, 464†, 644\*  
 —in serum, during testosterone therapy for carcinoma of the breast, 74, 423\*  
 —lactate, in the treatment of parathyroid insufficiency, 433\*  
 —metabolism in hyperparathyroidism with myxedema, 152\*  
 —renal calcinosis and calculi; effect of hypertonic saline infusions on urine flow in, 753\*

CANCER: *see* Tumors, and under various endocrine glandsCANCER RESEARCH CONGRESS, fourth international: *see* Association, newsCARBOHYDRATE METABOLISM: *see also* Diabetes mellitus

- appetite for carbohydrate in normal and hyperthyroid rats, determined by taste-threshold method, 467†  
 —carbohydrate tolerance; physiologic changes in, during pregnancy, 230

CARBOHYDRATE METABOLISM  
(cont.)

- dextrose tolerance: } *see* Carbohydrate
- Exton-Rose test: } metabolism, su-
- glucose tolerance: } gar tolerance
- glucose in treatment of diabetic ke-  
tosis, 229
- glycogenic corticosteroids in urine,  
in endocrine and other disorders, 79\*,  
331\*, 729†
- effect of testosterone on excretion  
of, 729\*
- hyperglycemia, paroxysmal, due to  
pheochromocytoma of adrenal, 30\*
- hypoglycemia, in infants, 533
- rapid test for detection of, 72
- severe, in Addison's disease, 134\*
- influence of oral ingestion of glucose  
and fructose on respiratory quotient  
and blood pyruvate and lactate re-  
sponses, with and without insulin, in  
diabetes mellitus, 472
- in pigeon; cyclic glycemias and  
lipemias associated with egg produc-  
tion, 661
- melituria in healthy American men  
with reference to transitory glyco-  
suria, 473
- sugar (dextrose, glucose) tolerance:  
Exton-Rose test, fallacy of, 232
- in virilism, 11\*
- two-dose, in diagnosis of diabetes  
mellitus and renal glycosuria, 229

CARBON TETRACHLORIDE, liver and  
gonadal changes following administra-  
tion to male rats and female guinea  
pigs, 448†

CARCINOMA: *see* Tumors; and under vari-  
ous endocrine glands

CASTRATION: *see* incidental to Clima-  
ctic; Menstruation; Ovary; Testis

CHAIRI-FROMMEL SYNDROME: *see*  
Syndrome

CHEMISTRY: *see* Methods; Tests; and  
under various chemical compounds

CHILDREN: *see also* Dwarfism; Growth;  
Infants; and under various glandular  
disorders

- Addison's disease in, 383
- basal metabolism in, 382
- craniopharyngiomas in, 376
- diabetic, growth of, in relationship to  
level of control of the disease, 727
- diabetic, insulin regulation in, 205\*
- gonadotropins, urinary, in prepu-  
beral children, 1\*
- menstruation in, 171\*
- tables for predicting height, 473

CHLORIDE: *see also* Addison's disease;  
Sodium

## CHLORIDE (cont.)

- excretion during glycosuria, in dia-  
betes, 231
- hypertonic saline infusions in dif-  
ferential diagnosis of diabetes in-  
sipidus and psychogenic polydipsia,  
464†, 753\*

## CHOLESTEROL:

- cholesterol, cholesterol esters and  
phospholipid phosphorus in blood in  
thyroid disease, 76
- content of adrenals, correlation with  
histological changes in various types  
of disease, 463†
- in blood, in hyperthyroidism; effect of  
treatment with Vitamin A, 574\*

CHORIONIC GONADOTROPIN: *see* Gon-  
adotropins; Pregnancy, diagnosis

CHROMAFFIN: *see* Pheochromocytoma;  
Tumors

CHROMOPHOBE ADENOMA: *see* Pitui-  
tary

CHRONIC DISEASE, effect on 17-ketos-  
teroid excretion in urine, 264\*

CIBA AWARD: *see* Association, awards

CIRCUMCISION: *see* Penis

CIRRHOSIS: *see* Liver

CLIMACTERIC: *see also* Androgen; Estro-  
gen; Menstruation; Ovary

- male, testosterone in treatment of  
chest discomfort in, 74
- urinary gonadotropins in, 1\*
- menopause, dienestrol therapy in,  
448†, 688\*
- hexestrol therapy in, 607
- menopausal exophthalmic goiter,  
treatment of, with massive doses of  
vitamin A, 574\*
- oral estrogen therapy in cases com-  
plicated by arthralgia, 451†
- psychiatric and endocrinological  
factors in, 226
- psychotherapeutic effect of estro-  
genic hormones, 228
- treatment with diethylstilbestrol  
dipalmitate; comparison of aqueous  
and oil preparations reabsorption  
and allergic reactions, 448†
- urinary gonadotropins in, 1\*

COBALT, with and without thyroxin; effect  
on blood regeneration following thy-  
roidectomy in rat, 306

COLD: *see also* Temperature

- extreme; effect on heat production in  
men, 73

CONGENITAL ANOMALIES: *see also*  
Hermaphroditism; and various glandular  
disorders

- absence of adrenals in case of Ad-  
dison's disease, 134\*

## CONGENITAL ANOMALIES (cont.)

- absence of one adrenal; tumor in other, 71
- anencephalic monster, 767\*
- associated with aplastic ovaries, sexual infantilism, high urinary gonadotropins and short stature, 665\*
- associated with "rudimentary ovary" syndrome, 385\*
- associated with short stature, retarded sexual development, no urinary gonadotropins, 807\*
- in offspring of a pituitary dwarf, 532

CORPUS LUTEUM: *see* OvaryCORTICOIDS: *see* Adrenals, steroidsCORTICOSTEROIDS: *see* Adrenals, steroidsCRANIOPHARYNGIOMA: *see also* Pituitary, tumors

- in children; evaluation of treatment; related lesions of hypophysis and hypothalamus, 376
- panhypopituitarism in patient followed 22 years after removal of craniopharyngioma, 79\*
- parapituitary tumors, craniopharyngiomas, 466†

CREATINE AND CREATININE, determination of uric acid-creatinine ration of ACTH, as test for adrenal cortex function, 459†

- excretion of, in urine following administration of ACTH, 458†, 459†
- storage and excretion of creatine; effect of thyroid, thiouracil, and of testosterone on, 381

CRETINISM: *see* ThyroidCRYPTORCHISM: *See also* Androgens; Eunuchoidism; Hermaphroditism; Hypogonadism; Testis

- bilateral, sublingual methyltestosterone therapy, 293\*

CUBITUS VALGUS: *see also* Congenital Anomalies

- associated with short stature, retarded sexual development and no urinary gonadotropins, 807\*
- in syndrome of congenitally aplastic ovaries with sexual infantilism, short stature, etc., 665\*

CUSHING'S SYNDROME: *see also* Adrenal; Pituitary

- adrenal cortical tumor in, 71, 331\*
- effect of bilateral hemiadenectomy; of testosterone; and of x-ray irradiation of pituitary on plasma protein pattern, 559\*
- effect of removal of adrenal cortical tumor; and of testosterone, on excretion of corticosteroids, 331\*, 729\*

## CUSHING'S SYNDROME (cont.)

- spontaneous remission followed by virilism; unusual case; autopsy, 787\*
- URINE: adrenocortical hormones, chemical assay of, 454†
- glyco-genic corticoids, 79\*, 729\*
  - gonadotropins, 1\*
  - 17-ketosteroids, 79\*, 331\*, 559\*, 729\*
  - 11-oxy-corticosteroids, 331\*
  - steroids, 543\*

DESOXYCORTICOSTERONE; acetate: *see* Adrenals, preparationsDEXTROSE TOLERANCE: *see* Carbohydrate metabolism; Diabetes mellitusDIABETES INSIPIDUS: *see* Pituitary, disordersDIABETES MELLITUS: *see also* Blood; Carbohydrate metabolism; Insulin

BLOOD, estimation of blood ketones in diabetes acidosis, 230

- influence of oral ingestion of glucose and fructose with and without insulin, on respiratory quotient and blood pyruvate and lactate responses, 472

- plasma protein pattern; in case complicated with acromegaly, 559\*

- sugar; two-dose dextrose tolerance test (Exton-Rose) in diagnosis, 229
- fallacy of, 232

- vitamin A and carotene metabolism as reflected by the blood levels in, 232

COMPLICATIONS AND SEQUELAE, acute pyelonephritis; and necrosis of renal papillae in, 471

- with acromegaly; disappearance of diabetes following acute mastoiditis and basilar meningitis, 455†

- with acromegaly; plasma protein pattern in, 559\*

- with chronic peptic ulcer, 779

- with hyperostosis frontalis interna, 624\*

- with hyperthyroidism; effect of treatment with thiouracil, 234

- with hyperthyroidism, melano-derma and amenorrhea, 303

- with hypopituitarism and hypoadrenalism (post-thyroidectomy), 376

- with pheochromocytoma; effect of removal of tumor, 716\*

EXPERIMENTAL, produced by alloxan; course of, in rat, 233

- produced by alloxan; effect of food intake on diabetogenic action of diethylstilbestrol, in rat, 449†

## DIABETES MELLITUS (cont.)

## EXPERIMENTAL (cont.)

- produced by alloxan; permanency of, and effect on pancreatic islets, in rat, 469†
- produced by diabetogenic hormone of anterior pituitary, 304
- produced by pituitary diabetogenic extract; effect on growth of normal animals, 75

## IN CHILDREN, behavior and psychological problems of young patients, 231

- growth of; in relationship to level of control of the disease, 727
- insulin regulation in diabetic children, 205\*

## IN PREGNANCY, effect of diabetic and prediabetic pregnancies on fetus and newborn infant, 728

- fetal mortality in women during prediabetic period, 727
- maternal, obstetrical, chemical, fetal and placental abnormalities, 471
- physiologic changes in carbohydrate tolerance in, 230
- treatment with insulin; effect on maternal and infant mortality, 230

## THERAPY, glucose and insulin therapy in diabetic ketosis, 229

- insulin regulation in diabetic children, 205\*
- regulation; analysis of 250 cases, 779
- treatment with insulin; effect on maternal and infant mortality, 230

## URINE, chloride excretion during glycosuria, 231

- glycogenic corticoid excretion in, 79\*, 729\*
- effect of testosterone on, 729\*
- 17-ketosteroid excretion in, 79\*, 729\*

DIENESTROL: *see* Estrogens

## DIET, effect on action of diethylstilbestrol upon alloxan diabetes, in rat, 449†

- effect on body weight, in internment camp, 71
- effect on growth and survival of adrenalectomized rats, 70
- in regulation of diabetes mellitus, analysis of 250 cases, 779
- low potassium, high chloride; in treatment of Addison's disease, 139\*

DIETHYLSTILBESTROL: *see* Estrogens

## DIHYDROTACHYSTEROL, in the treatment of parathyroid insufficiency, 433\*

## DIIDOTYROSINE, synthesis, as studied by transformation of radioactive iodine, 235\*

## DIODRAST, renal plasma clearance of, in hyperthyroidism and myxedema, 801\*

DWARFISM: *see also* Children; Growth; Ovary, syndrome: Pituitary

- associated with congenital defects, retarded sexual development, no urinary gonadotropins, 807\*
- associated with congenitally aplastic ovaries, sexual infantilism, high urinary gonadotropins and other congenital abnormalities, 665\*
- associated with ovarian agenesis, 11\*
- associated with primary amenorrhea, 609
- moderate, in "rudimentary ovary" syndrome; comparison with hypophyseal dwarfism, 385\*
- pituitary; pregnancy in; congenital abnormalities of fetus, 532

DYSMENORRHEA: *see* Menstruation

## EARS, auricular hairs: relation to race, endocrine stimulation and urinary steroids, 465†

## EDEMA, during estrogen therapy for dysmenorrhea, 450†

EDITOR, letters to: *see* Association, lettersENDOCRINE: *see also* Androgens; Estrogens, Hormones; Steroids; and under various endocrine glands

- diseases; chemical assay of adrenocortical hormones in urine, 454†
- disorders, associated with Cushing's syndrome, followed by virilism; unusual case, 787\*
- disorders; urinary glycogenic corticoids and 17-ketosteroids in, 79\*
- endocrines in gynecology, 384
- endocrinopathies; three unusual, associated with ovarian pathology, 11\*
- endocrinopathies; urinary gonadotropins in, 1\*
- studies, in a case of ovotestis, 741\*
- therapy, in pseudohermaphroditism; social and psychological readjustment, 456†

## ENDOCRINOLOGY, contribution of, to obstetrics and gynecology, 607

- postgraduate course in: *see* Association, 777

ENZYMES: *see under* name of enzymeEPINEPHRINE: *see* Adrenals, preparations

## ERGOT, synthetic: methergine, induction of labor, with, 606

ESTRADIOL: *see* EstrogensESTRIOL: *see* EstrogensESTROGENS: *See also* Climacteric; Menstruation; Ovary; Pregnancy; Steroids

- effect of chemical conjugation, vehicle, and route of administration of

## ESTROGENS (cont.)

- estrogen upon nature and amount of its excretion, 452†
  - endogenous; cervical cornification count as index of, 749\*
  - estrogen-androgen relationships in human organism, 453†
  - estrogenic insufficiency; in syndrome of rudimentary ovaries with increase in gonadotropins, 385\*
  - metabolic pathway of estriol production in the organism, 453†
  - natural; metabolism of single therapeutic dose:
    - comparison on gonadless female and male, 364\*
    - during menstrual cycle, 364\*
    - in human male, 452†
  - similarity of estrogen to hormone from Sertoli cells of testis, 493\*
  - tests for: comparison of color chemical test for pregnanediol with Friedman test, 608
    - estimation of urinary pregnanediol by liquid chromatogram technique, 457†
    - estrogenic steroids; simple quantitative colorimetric method for, 701\*
    - photometric estimation of estrogens in urine, 364\*, 452†
    - simple, quantitative, colorimetric for estrogens, 452†
- EFFECTS, action of, on bone; relationship of parathyroid glands to, 383
- and thiouracil; effect of, on lactogenic hormone and weight of pituitary in rat, 531
  - effect of diethylstilbestrol on glucuronidase activity in serum during normal and toxemic pregnancy, 535\*
  - effect of diethylstilbestrol on urinary gonadotropin excretion, 1\*
  - effect of food intake on diabetogenic action of diethylstilbestrol, in rat, 449†
  - effect of pregnanalone on excretion of 11-oxycorticosteroids in adrenal virilism, 331\*
  - effect of pregnenolone  $\Delta^5$  on spermatogenic activity, 227
  - effect of progesterone on endometrium in monkey, 611\*
  - effect of stilbestrol on high urinary gonadotropin output of syndrome of ovarian aplasia, sexual infantilism and short stature, 665\*
  - effect of stilbestrol on luteal phase of menstruation; in experimental alteration of human ovarian cycle, 450†

## ESTROGENS (cont.)

## EFFECTS (cont.)

- effect of topical application, on developmental separation of prepuce from glans penis, 192\*
  - effect on excretion of 11-oxycorticosteroids in adrenal virilism, 331\*
  - effect on excretion of pregnanediol in urine, 457†
  - increased excretion of pregnanediol in pregnancy from, 609
  - influence on endometrium, in the monkey, 711\*
  - inhibitory action of dienestrol on pituitary, 688\*
- IN URINE, decreased, in "rudimentary ovary" syndrome with increase of gonadotropins, 385\*
- distribution of, after ingestion of single therapeutic dose, 364\*
  - effect of dienestrol therapy on urinary estrogens, 688\*
  - effect of stilbestrol and of progesterone on excretion of 11-oxycorticosteroids, 331\*
  - estrogen-androgen relationships in human organism, measured by Zimmermann and Kober reactions applied concomitantly to urine, 453†
  - estrone clearance test, in infectious hepatitis, pregnancy, acromegaly, and cirrhosis of liver, 519\*
  - excretion, effect of chorionic gonadotropin therapy on, 446†
  - in case of congenital defects, short stature, retarded sexual development and no urinary gonadotropins, 807\*
  - in feminizing testicular tumor, 438\*
  - in gonadless female: compared with human male, 364\*
  - in male gynecomastia, 586\*
  - in precocious puberty, 171\*
  - in virilizing ovarian tumor, 115\*
  - marked increase in excretion following administration of carbon tetrachloride to female guinea pigs, 448†
  - metabolism of therapeutic doses, in human male, 452†
  - pregnane derivative, in precocious puberty, 171\*
  - pregnanediol excretion; antepartum and postpartum, 608
  - pregnanediol excretion in late pregnancy, increased by diethylstilbestrol, 609
  - pregnane-diols and -triols in urine in adrenal cortical tumors and hyperplasia, 543\*

## ESTROGENS (cont.)

THERAPY, as a psychotherapeutic agent, 228

- cause of oligospermia and of decreased excretion of 17-ketosteroids, in normal male, 186\*
- dienestrol; evaluation of, in treatment of amenorrhea in young women; the menopause; inhibition of lactation; and various gynecological conditions, 448†, 688\*
- diethylstilbestrol dipalmitate in aqueous suspension, in treatment of menopause; absorption of; and freedom from local allergic reactions following administration, 448†
- effect of treatment with, in a case of congenital defects, short stature, retarded sexual development, and no urinary gonadotropins, 807\*
- effect on hydrogen ion concentration of senile vaginal mucosa, 226
- hexestrol in treatment of estrogen deficiency, 607
- in treatment of hyperthyroidism, 380
- in treatment of syndrome of ovarian aplasia, sexual infantilism, high urinary gonadotropins, and short stature, 665\*
- oral, during menopause; and when complicated by arthralgia, 451†
- progesterone; high dosage combined with estrogen, in treatment of amenorrhea and of abortion, 609
- in treatment of precocious puberty, 171\*
- in treatment of uterine fibromyomata, 446
- stilbestrol, effect on secondary sarcomatous deposits in the lungs, 226
  - for engorgement and lactation in nonnursing mothers, 607
  - in treatment of malignant exophthalmos by suppression of pituitary function, 102\*
  - in treatment of ovarian agenesis, 11\*
  - in treatment of virilizing ovarian tumor, 115\*

EUNUCHOIDISM: *see also* Androgens; Hypogonadism; Sterility; Testis

- associated with gynecomastia, 586\*
- failure to respond to chorionic gonadotropic hormone, due to antihormones, 130\*
- hypogonadotropic; urinary gonadotropins in, 1\*

## EUNUCHOIDISM (cont.)

- "pan hypopituitary" eunuchoid; spermatogenesis in, as result of testosterone therapy by pellet implantation, 781\*
- prepuberal, with hyperthyroidism, 566\*
- treatment with combination of testosterone and pregnenolone, 227
- treatment with sublingual methyltestosterone, 293\*

ESTRONE: *see* Estrogens

EXOPHTHALMIC GOITER: *see* Thyroid, goiter

EXOPHTHALMOS: *see* Eyes; Thyroid, goiter

EXTON-ROSE sugar tolerance test: *see* Carbohydrate metabolism; Diabetes mellitus; Tests

EYES, cataracts following postoperative parathyroid insufficiency, 433\*

- changes in prominence, in various thyroid states, 306
- conjunctival and corneal lesions in hypercalcemia, 464†, 644\*
- corneal epithelium of rat; effect of thiouracil on mitotic activity and wound healing, 468†
- exophthalmos, following administration of the thyrotropic hormone of the pituitary gland, to guinea pig, 305
  - intractable; surgery of, 379
- hyperophthalmopathic syndrome (malignant exophthalmos), in thyroid disease; treatment, 102\*
  - urinary 17-ketosteroids, gonadotropins, and estrogens in, 102\*
- improvement in vision during treatment of polyostotic fibrous dysplasia with testosterone, 455†
- intraocular transplants of endometrium, in study of menstrual cycle; influence of ovarian hormones on, in the monkey, 611\*

FACE, stimulation of growth of mandible by oral use of crude adrenal cortex extract, 457†

FAMILIAL: *see also* under various endocrine glands

- Addison's disease, 134\*
- adrenal cortex disorder, 543\*, 604
- eunuchoidism, 293\*
- goiter and cretinism in brothers, 77
- Lawrence-Moon-Biedl syndrome, 780
- pheochromocytoma, bilateral, 475\*

FAT METABOLISM: *see also* Obesity

- changes in, in guinea pig, produced by thyrotropic hormone of anterior pituitary, 305



## FAT METABOLISM (cont.)

- in pigeon; cyclic glycemias and lipemias associated with egg production, 661
- lipid distribution in adrenal cortex, a measure of functional activity, 463†
- new hormone of adrenal cortex; relation to fat metabolism in liver, 461†

FERTILITY: *see* Ovary; Pregnancy; Sterility; Testis

FETUS, effect of diabetes complicating pregnancy upon, 471

- effect of diabetic and prediabetic pregnancies on, 728
- effect of thiouracil on derivatives on, 47\*, 381, 469†, 767\*
- effect of thiouracil upon iodine content of thyroid gland of anencephalic monster, 767\*
- fetal mortality in women during prediabetic period, 727, 728
- mortality in pregnant diabetics; effect of insulin on, 230
- prenatal function of human parathyroid glands, 383
- sex of; correlation of blood gonadotropins and vaginal smears of mother with, 450†
- size and shape: relation to hydrostatic condition of uterus, 474

FIBROMYOMA: *see* Tumors; Uterus

FOLLICLE-STIMULATING HORMONE (F.S.H.): *see* Gonadotropins; Pituitary

FROG, SOUTH AFRICAN, use of, in diagnosis of hydatidiform mole by urinary gonadotropin assay, 289\*

GASTRO-INTESTINAL TRACT, chronic peptic ulcer associated with diabetes, 779

- duodenal ulcer, hypertrophy and hyperplasia of parathyroid glands associated with, 383

GLUCOSE: *see* Carbohydrate metabolism; Diabetes mellitus

GLUCURONIDASE activity in serum during normal and toxemic pregnancy, 535\*

- effect of diethylstilbestrol on, in normal puerperium, 535\*
- in various body tissues, 535\*

GLYCOSURIA: *see also* Carbohydrate metabolism; Diabetes mellitus

- transitory; relation to melituria in healthy American men, 473

GOITER: *see* Thyroid

GOITER SOCIETY: *see* American Association for the Study of Goiter in: Association, news.

GONAD: *see also* Androgens; Estrogens; Menstruation; Ovary; Pregnancy; Steroids; Testis

## GONAD (cont.)

- atrophy in myotonia dystrophica, 503
- function as indicated by 17-ketosteroid excretion in various conditions associated with mental retardation, 503\*
- gonadal stimulation following administration of antigonadotropic serum to rats, 447†
- liver and gonadal changes following administration of carbon tetrachloride to male rats and female guinea pigs, 448†
- ovotestis, effect of removal upon urinary gonadotropins and 17-ketosteroids, 455†
- sex steroids; relationship to weight of adrenal glands of hamsters and rats, 463†

GONADOTROPINS: *see also* Pituitary; Pregnancy; Urine

- basophilism and decrease in gonadotropin content of pituitary following administration of antigonadotropic serum to rats, 447†
- chorionic, effect on pregnanediol excretion, 446†
  - effect of spermatogenic activity in toad, 653\*
  - failure to respond to, in eunuchoidism, due to antihormones, 130\*
- distortion of spiral artery and hemorrhagic cysts of ovary in rabbit, after administration of, 447†
- equine pituitary; development of antihormones, 466†
- gonadal stimulation following administration of antigonadotropic serum to rats, 447†
- in blood; changes in, during pregnancy in correlation with the fetal sex, 450†
- treatment of "pan-hypopituitary" eunuchoid with pregnant mare serum, 781\*

IN URINE, absence of, in case of congenital defects, short stature and retarded sexual development, 807\*

- chemistry: comparison of ultrafiltration and alcohol-precipitation methods in normal subjects and in various endocrinopathies, 1\*

- chorionic: assay, using South African frog; in diagnosis of hydatidiform mole, 289\*

- determination of, by hyperemia AZT: evaluation of hyperemia rat unit; in diagnosis of pregnancy, 159\*

## GONADOTROPINS (cont.)

## IN URINE (cont.)

- during progesterone therapy of uterine fibromyomata, 446†
- effect of dienestrol therapy on, 688\*
- effect of removal of ovotestis on, 455†
- high, in primary amenorrhea associated with short stature and sexual infantilism, 609
- high titer in bilateral arrhenoblastoma without masculinization; large breasts, 456†
- in a case of ovotestis, 741\*
- in azoospermia without impairment of Sertoli or Leydig cells of testes, 493\*
- in acromegaly, 636\*
- in azoospermatic testicular syndrome, 493\*
- in eunuchoidism, 130\*, 781\*
- in feminizing tumor of testis, 438\*
- in gigantism, 1\*
- in hypogonadism, 466†
- in male gynecomastia, 586\*
- in ovarian agenesis, 11\*
- in "pan-hypopituitary" eunuchoidism, 781\*
- in precocious puberty, 11\*, 171\*
- in virilism, 11\*
- in virilizing ovarian tumor, 115\*
- increased in "rudimentary ovary" syndrome, 385\*
- increased in syndrome of congenitally aplastic ovaries with sexual infantilism, etc.; effect of estrogens upon, 665\*

GONOCYTOMA: *see* Ovary, tumor; Testis, tumor

GRAVES' DISEASE: *see* Thyroid

GRID baby, for measurement of growth and development of infants, 780

GROWTH: *see also* Children; Dwarfism; Infants

- effect of diet on, in adrenalectomized rats, 70
- effect of liver feeding on, in hyperthyroid rat, 532
- effect on basal metabolism, 382.
- in infant, measured by grid technique, 780
- macrogenitosomia with extreme rapidity of growth, in congenital adrenal-cortical insufficiency, 604
- of children with diabetes mellitus, in relationship to level of control of the disease, 727
- tables for prediction of height of children, 473

GYNECOMASTIA: *see* Breast

GYNECOLOGY: *see also* Climacteric; Menstruation; Ovary; Pregnancy; Uterus; Vagina

- contributions of endocrinology to, 607

HAIR: *see also* Eunuchoidism; Hermaphroditism; Hypogonadism; Virilism; and various endocrine gland disorders

- absence of, in case of retarded sexual development, short stature, congenital defects and no urinary gonadotropins, 807\*
- absence of sexual hair in ovarian agenesis, 11\*, 665\*
- alopecia totalis in case of virilizing ovarian tumor; effect of removal of tumor, 115\*
- auricular hairs; relation to race, endocrine stimulation and urinary steroids, 465†
- axillary; amount of, as indicator of androgenic function of adrenal, 52\*
- hypotrichosis axillaris in thyrotoxicosis, telangiectasia of the skin, liver palms, and gynecomastia, 52\*
- sexual, in "rudimentary ovary" syndrome, 385\*

HIRSTUTISM, associated with arrhenoblastoma, 606, 607

- associated with hyperostosis frontalis interna, etc., 624\*
- caused by testosterone propionate in woman treated for carcinoma of breast, 423\*
- decrease after removal of arrhenoblastoma, 606
- excretion of glyco-genic corticoids in, 79\*
- excretion of gonadotropin in, 1\*
- excretion of 17-ketosteroid in, 79\*, 331\*
- excretion of 11-oxy-corticosteroids, 331\*
- in a case of ovotestis, 741\*
- in Cushing's syndrome: regression; recurrence, with reappearance of virilism, 787\*
- in patient with aberrant adrenal cortex tissue, 385\*
- in precocious puberty, 11\*
- in virilism, 11\*
- in virilizing ovarian tumor; effect of removal of tumor, 115\*

HEALING, wound healing and mitotic activity in corneal epithelium of rats treated with thiouracil, 468†

HEART, angina-pectoris; lack of effect of testosterone in, 74

- cardiotropic activity of anterior pituitary in thyroidectomized rats, 303

**HEART** (cont.)

- congestive failure; effect of 2-thiouracil in, 74
- differential diagnosis of myxedema and heart disease, 468†
- thyrocardiac disease; value of thyroidectomy in, 379

**HEAT PRODUCTION:** *see also* Temperature

- in men, during extreme cold, 73

**HEIGHT:** *see* Dwarfism; Growth**HEMOCHROMATOSIS**, without pigmentation or diabetes, 72**HERMAPHRODISM:** *see also* Ovary; Testis

- effect of removal of ovotestis on urinary gonadotropin and 17-ketosteroids in, 455†
- frequency of inguinal hernia in, 741\*
- ovotestis; endocrine studies, 455†, 741\*
- pseudo; social and psychological readjustment under endocrine therapy, 456†
  - urinary gonadotropins in, 1\*
  - with mental changes, effect of adrenalectomy upon, 533
- sublingual methyltestosterone therapy, 293\*
- urinary steroids in, 543\*

**HERNIA**, inguinal, in relation to hermaphroditism, 741\***HEXESTROL:** *see* Estrogens**HIRSUTISM:** *see* Hair**HISTAMINE** in diagnosis of pheochromocytoma (Roth-Kvale test), 475\***HORMONE:** *see also* Androgens; Estrogens; Gonadotropins; Steroids; and various endocrine glands

- new hormone of adrenal cortex; relation to fat metabolism in liver, 461†
- new testicular hormone, similar to estrogen, 493\*

**HYDATIDIFORM MOLE:** *see also* Pregnancy

- diagnosis, using South African frog for assay of chorionic gonadotropin, 289\*
- relation to eclampsia, 605

**HYDROPONICS**, used to produce iodine-free diet, 468†, 714\***HYPERCALCEMIA:** *see* Blood; Calcium metabolism**HYPEREMIA** rat unit of chorionic gonadotropin, evaluation of, 159\***HYPERGLYCEMIA:** *see* Carbohydrate metabolism; Diabetes mellitus**HYPEROPHTHALMOPATHIC** syndrome in thyroid disease; treatment, 102\***HYPEROSTOSIS**, cranialis interna in case of virilizing ovarian tumor, 115\***HYPEROSTOSIS** (cont.)

- frontalis interna syndrome, not a clinical entity, 624\*

**HYPERTENSION**, associated with hyperostosis frontalis interna, etc., 624\*

- associated with menopausal exophthalmic goiter; effect of vitamin A, 574\*
- development of goiter and myxedema in patient treated for hypertension with potassium thiocyanate, 235\*, 244
- in toxemia of pregnancy; serum glucuronidase in, 535\*
- malignant, produced by medullary suprarenal chromaffinoma, 533
- paroxysmal, associated with pheochromocytoma, 30\*, 475\*, 534, 716\*
  - produced by histamine, 475\*

**HYPERTHYROIDISM:** *see* Thyroid**HYPERTRICHOSIS:** *see* Hair, hirsutism**HYPOGLYCEMIA:** *see* Blood; Carbohydrate metabolism; Diabetes mellitus**HYPOGONADISM:** *see also* Eunuchoidism; Gonad; Ovary; Sterility; Testis

- male; evaluation of urethral smear test for androgenic deficiency; effect of androgen therapy, 186\*
- sublingual methyltestosterone therapy, 293\*

**HYPOTHALAMUS:** *see also* Pituitary

- lesions of hypophysis and hypothalamus, related to craniopharyngioma in children, 376

**HYPOTHYROIDISM:** *see* Thyroid**IMPLANTATION:** *see* Pellet implantation**INFANTILISM:** *see* Dwarfism; Ovary; Pituitary**INFANTS**, effect of topical application of steroid hormones on developmental separation of prepuce from glans penis, 192\*

- growth and development: measured by grid technique, 780
- hypofunction of adrenals; treatment with desoxycorticosterone acetate, 533
- newborn, effect of diabetic and pre-diabetic pregnancies on, 727, 728
- premature, treatment with methyltestosterone and testosterone propionate, 465†, 708\*

**INFECTIONS**, disappearance of diabetes mellitus associated with acromegaly, following acute mastoiditis and basilar meningitis, 455†

- effect on urinary cortin, 70, 70
- infectious mononucleosis; arginase activity of red blood cells in, 326\*
- typhoid vaccine in assay of protective power of adrenal extracts and steroids, 69

INFERTILITY: *see* Sterility

INSULIN, crystalline; absorption of pellets and solutions, in rabbits, 233

— in regulation of diabetes mellitus; analysis of 250 cases, 779

— in treatment of diabetic children, 205\*

— in treatment of diabetic ketosis, 229

— in treatment of pregnant diabetics; effect on infant and maternal mortality, 230, 230

— tolerance tests in pituitary tumors, 466†

— tolerance test, in "rudimentary ovary" syndrome, 385\*

INULIN, renal plasma clearance of, in hyperthyroidism and myxedema, 801\*

IODINE: *see also* Thyroid

— and adrenalin; effect on action of thyrotropin in Graves' disease, 532

— in blood, in eunuchoidism with hyperthyroidism, 566\*

— in blood, in obesity, 462†

— in human fetal thyroid: effect of thiouracil therapy in mother, 47\*, 469†, 767\*

— influence on action of vitamin A in hyperthyroidism, 574\*

— iodine-free diet grown by hydroponies and excluding goiter noxa, production of goiter on, 714\*

— iodine-lack theory and endemic goiter, 58\*, 60\*, 62\*, 820\*

— severe iodism complicating thyrotoxicosis; cutaneous sensitivity tests, 212\*

RADIOACTIVE, in study of metastatic thyroid carcinoma, 467†

— in treatment of Graves' disease; 78, 378

— in treatment of hyperthyroidism, 235\*, 377

— in fractionation studies of pathological thyroid tissue, 380

— radio iodine; its use as a tool in study of thyroid physiology; balance studies, 235\*

— excretion in urine by patients with struma medicamentosa and with Graves' disease, 235\*

— collection by thyroids of patients with Graves' disease, 235\*

IRRADIATION, of pituitary, in Cushing's syndrome, effect on plasma protein pattern, 559\*

— irradiation of pituitary in hyperophthalmopathic syndrome in thyroid disease, 102\*

— radiation therapy in hyperthyroidism, 380

IRON; effect on anemia produced by hypophysectomy in rats, 376

JAUNDICE: *see* Liver

KETOSIS: *see* Diabetes mellitus

17-KETOSTEROIDS: *See* Androgens; Steroids; Urine; and under various endocrine glands

KIDNEY, hepato-renal factors in circulatory homeostasis; relation of adrenals to formation of renal vaso-exeitor principle, 460†

— impaired renal function in pituitary hypothyroidism, 74

— necrosis of renal papillae; and acute pyelonephritis in diabetes mellitus, 471

— renal insufficiency, renal calculi and renal calcinosis; effect of hypertonic saline solutions in, 753\*

— renotropic activity of anterior pituitary in thyroidectomized rats, 303

— specific renal functions in hyperthyroidism and myxedema, 801\*

KOBER REACTION: *see* Tests

LACTATION: *see* Breast; Estrogens, therapy

LAURENCE-MOON-BIEDL SYNDROME: *see* Syndrome

LAURENTIAN HORMONE CONFERENCE: *see* Association, news

LETTERS TO THE EDITOR: *see* Association, letters

LEUKEMIA: *see* Blood

LEUKOCYTES: *see* Blood

LEUKODERMA: *see* Pigmentation

LIPIDASE, in blood; effect of ligation of pancreatic ducts on, in cats, 231

LIVER, cholelithiasis in Addison's disease, 134\*

— cirrhosis of; estrone clearance in, 519\*

— cirrhosis; pathogenesis of, in patients with diffuse toxic goiter, 379

— effect on growth and ovarian development in hyperthyroid rats, 532

— "fat factor" of the adrenal in relation to fat reserves of the liver, 461†

— gonadal and liver changes following administration of carbon tetrachloride to male rats and female guinea pigs, 448†

— hemochromatosis without pigmentation or diabetes, 72

— hepatitis, infectious, in pregnancy: diagnosis of; estrone clearance in, 519\*

— hepato-renal factors in circulatory homeostasis, 460†

## LIVER (cont.)

- jaundice, during aminothiazole therapy in thyrotoxicosis, 812\*
- jaundice, fatal, resulting from thiouracil, 830\*
- "liver palms" in thyrotoxicosis; relation of hypotrichosis axillaris to, 52\*

LUNGS; secondary sarcomatous deposits in; effect of stilbestrol on, 226

MACROGENITOSOMIA: *see* Adrenals, diseases; Puberty, precocious

MALE CLIMACTERIC: *see* Climacteric

MALNUTRITION: *see* Anorexia nervosa; Diet; Growth; Weight

MANDIBLE: stimulation of growth by oral use of crude adrenal cortex extract, 457†

MARIE-STROMPELL'S DISEASE: *see* Arthritis

MASTITIS: *see* Breast

MELANODERMA: *see* Pigmentation

MELITURIA in healthy American men, relation to transitory glycosuria, 473

MENOPAUSE: *see* Climacteric

MENSTRUATION: *see also* Estrogens; Ovary; Puberty, precocious; Uterus

AMENORRHEA, associated with Cushing's syndrome: spontaneous remission and normal menstruation; followed by amenorrhea with appearance of virilism, 787\*

— caused by treatment with testosterone propionate for carcinoma of breast, 423\*

— dienestrol therapy in, 688\*

— due to uterine atrophy associated with hyperthyroidism, 303

— in case of retarded sexual development, short stature, congenital defects, no urinary gonadotropins, 807\*

— in young women, treatment with dienestrol, a synthetic estrogen, 448†

— primary, associated with short stature, sexual infantilism and high gonadotropins, 609

— primary, in "rudimentary ovary" syndrome, 385\*

— primary, in syndrome of congenitally aplastic ovaries, sexual infantilism, high urinary gonadotropins and short stature, 665\*

— progesterone therapy, high dosage, combined with estrogen therapy, 609

— result of granulosa-cell tumor, 605

— analysis of 257 cases of sterility, 610

— corpus luteum function, 446†

## MENSTRUATION (cont.)

## AMENORRHEA (cont.)

— *cycle*; alteration in, through administration of stilbestrol, 450†

— comparison of vaginal and cervical cornification in, 749\*

— correlation of body temperature curves with endometrial biopsy, 451†

— distribution of estrogens in urine during the menstrual cycle, 364\*

— dysmenorrhea; effect of stilbestrol on, 450†

— effect of estrogen on luteal phase of menstruation, 450†

— effect of hypothyroidism on, 467†

— experimental; factor of previous treatment in, 449†, 611\*

— experimental; structure of endometrial vascular bed in menstrual cycle in monkey; influence of ovarian hormones on, 449†, 611\*

— in children, 171\*

— menorrhoeal problems in college women; treatment, 610

— menstrual disorders associated with hyperostosis frontalis interna, etc., 624\*

— menstrual misbehaviors; psychic factors in, 659\*

— ovulation, delayed, in monkey, 609

— ovulation time in the monkey, 384

— plasma levels and urinary excretion of ascorbic acid during menstrual cycle, 607

— pregnandiol precipitation test in various disorders of, 351\*

— pre-menstrual tension; vitamin A therapy, 574\*

MENTAL development and disorders: *see* Brain; Congenital anomalies; Psychic factors; Psychosis

META-DINITROBENZENE REACTION, in determining chromogenicity of steroids, 454†

METHERGINE, induction of labor with, 606

METHODS: *see also* Tests

— baby grid for measurement of growth and development of infants, 780

— chemical assay of urine for adrenocortical hormones, in endocrine and non-endocrine diseases, 454†

— comparison of ultrafiltration and alcohol-precipitation methods for determination of urinary gonadotropin, 1\*

— direct photoelectric, for determination of serum calcium, 73

— for estimation of sodium in body fluids, 72

## METHODS (cont.)

- for studying vaginal and cervical cornification, 749\*
- rapid, for detection of hypoglycemia, 72
- rapid, for determination of 17-ketosteroids in urine, 451†, 795\*
- simple quantitative colorimetric for estrogenic steroids, 701\*
- specific clearance, for renal functions, 801\*
- tables for predicting height from skeletal age and present height of children, 473
- Tiselius electrophoretic technique used to determine plasma protein pattern, 559\*

METHYL TESTOSTERONE: *see* Androgens

METHYLTHIOURACIL: *see* Thiouracil

MISCARRIAGES: *see* Pregnancy

MITOTIC ACTIVITY and wound healing in corneal epithelium of rats treated with thiouracil, 468†

MONGOLISM, 17-ketosteroids in urine, 503\*

MUSCLE in acromegaly, associated with amyotrophic lateral sclerosis and acromegaly of the amyotrophic type, 636\*

- myotonia dystrophica; marked decrease of 17-ketosteroids in urine and gonadal atrophy in, 503\*

MYOTONIA DYSTROPHICA: *see* Muscle

MYXEDEMA: *see* Thyroid

NECK, webbed in syndrome of congenitally aplastic ovaries with sexual infantilism, short stature, etc., 665\*, 807\*

NEGRO, Addison's disease in, 69

NEOPLASMS: *see* Tumors

NERVOUS SYSTEM: *see also* Brain; Psychic factors

- acromegaly associated with amyotrophic lateral sclerosis and acromegaly of the amyotrophic type, 636\*
- role of, in autotransplantation of thyroid, 75

NOTICES: *see* Association, announcements

NUTRITION: *see* Anorexia nervosa; Diet; Growth; Weight

OBESITY: *see also* various disorders such as Cushing's syndrome, etc.

- associated with hyperostosis frontalis interna, etc., 624\*
- blood, iodine in, 462†
- emotional factors in, 225
- treatment of, 225, 462†
- urine, steroids in, 462†

OBITUARY, Laqueur, Ernst, 603

OBSTETRICS AND GYNECOLOGY, contributions of endocrinology to, 607

OLIGOPHRENIA: *see* Brain

OLIGOSPERMIA: *see* Testis, spermatozoa

OVARY: *see also* Breast; Climacteric; Estrogens; Gonad; Gonadotropins; Hair; Hermaphroditism; Menstruation; Pregnancy; Puberty, precocious; Sex; Tumors; Uterus; Vagina

## DISORDERS

- agenesis: *see* Ovary, syndrome
- amenorrhea: *see* Menstruation
- analysis of 257 cases of sterility, 610
- arrhenoblastoma: *see* Ovary, tumors
- climacteric: *see* Climacteric
- contributions of endocrinology to gynecology and obstetrics, 384, 607
- endocrinopathies, three unusual: ovarian agenesis; precocious puberty, and virilism; ovarian pathology and urinary gonadotropins in, 11\*
- female surgical castrate; urinary excretion of estrogens in, compared with that of human male, 364\*
- hermaphroditism: *see* Hermaphroditism
- hermaphroditism: a case of ootestis, 741\*
- menopause: *see* Climacteric
- menstrual disorders: *see* Menstruation
- precocious puberty: *see also* Puberty, precocious
- precocious puberty: constitutional type: effect of unilateral ovariectomy in, 171\*
- syndrome
  - ovarian agenesis, short stature, high urinary gonadotropins, 11\*
  - congenitally aplastic ovaries with sexual infantilism, high urinary gonadotropins, short stature and other congenital abnormalities, 665\*
  - primary amenorrhea, short stature, sexual infantilism, high gonadotropins, 609
  - retarded sexual development associated with congenital defects, short stature, no urinary gonadotropins, 807\*
  - rudimentary ovaries with estrogenic insufficiency and increase in gonadotropins, 385\*
- therapy: *see* Estrogens
- urine in: *see* Estrogens in urine; Urine

## OVARY (cont.)

- PHYSIOLOGY, atrophy following administration of carbon tetrachloride to female guinea pigs, 448†
- corpus luteum- function, studies, 446†
  - cyclic glycemias and lipemias associated with egg production in pigeon, 661
  - development, effect of liver feeding on, in hyperthyroid rat, 532
  - distortion of spiral artery and corpus hemorrhagicum cysts following administration of gonadotropins to rabbits, 447†
  - function; effect of vitamin A therapy on, 574\*
  - marked ovarian and uterine stimulation following administration of antigonadotropic serum, in rat, 447†
  - menstruation: *see* Menstruation
  - normal human female urine; pituitary adrenocorticotrophic substance in; effect on ascorbic acid level in adrenal, 71
  - ovulation and conception; test for determining time of, 606
  - ovulation: correlated with viscosity of cervical mucus and body temperature, 610
    - delayed, in monkey, 609
    - time in the monkey, 384
  - pregnancy: *see also* Pregnancy
  - reaction of ovary to chorionic gonadotropin in new hyperemia AZ test, 159\*

PREPARATIONS: *see* Estrogens, therapy.

## TUMORS

- adrenal-like tumor of, 605
- arrhenoblastoma, adrenal-like ovarian tumor, a variant of, 115\*
- arrhenoblastoma—androblastoma 228
  - bilateral, without masculinization; large breasts: high urinary gonadotropins (adenoma testiculare of Piek), 456†
  - excretion of hormones in, 115\*
  - excretion of 17-ketosteroids and pregnanediol in, 607
  - results of treatment, 115\*, 606, 606, 607
- gonocytoma, 227
- granulosa-cell tumor; pregnancy following removal, 605
- granulosa-cell tumor in 55 year old woman, 605
- homologous ovarian and testicular tumors, 227, 228

## OVARY (cont.)

## TUMORS (cont.)

- review of literature, 226
- virilizing tumors of, 115\*

PANCREAS: *see also* Diabetes mellitus; Insulin

- diagnosis of chronic pancreatic disease, non-specificity of serum amylase and lipidase in, in cats, 231
- effect of ligation of ducts, on amylase and lipidase content of blood, in cats, 231

PANHYPOPITUITARISM: *see* Eunuchoidism; Pituitary

PARATHYROID: *see also* Calcium metabolism

## HYPERPARATHYROIDISM

- aberrant adenoma of parathyroid; effect of removal, 152\*
- associated with myxedema and psychosis, 152\*
- calcium and phosphorus metabolism in, 152\*
- conjunctival and corneal lesions in, 644\*
- gastro-intestinal symptoms in, 152\*
- hypertrophy and hyperplasia of, associated with duodenal ulcer, 383
- insufficiency, postoperative, followed by cataracts; symmetric cerebral calcification; treatment, 433\*
- prenatal function of, in human subject, 383
- relationship of, to the action of estrogen on bone, 383

## PELLET IMPLANTATION

- desoxycorticosterone; in adrenalectomized rats; effect of diet on, 70
- desoxycorticosterone acetate in treatment of Addison's disease, 134\*
- desoxycorticosterone acetate; in treatment of cortex insufficiency, 331\*, 462†
- insulin, zinc crystalline; absorption of, in rabbits, 233
- progesterone; in therapy of fibromyomata of uterus, 446†
- testosterone; in treatment of eunuchoidism, 781\*
  - in treatment of male hypogonadism, 293\*, 781\*

PENIS: *see also* incidental to disorders of Testis; effects of Androgens; Gonadotropins

- glans, effect of topical application of steroid hormones upon developmental separation of prepuc from, in human infants and in squirrels, 192\*

**PHEOCHROMOCYTOMA**, a case, 534

- bilateral, familial, with paroxysmal hypertension; goiter associated with; successful surgical removal, 475\*
- medullary suprarenal chromaffinoma producing malignant hypertension, 533
- of right adrenal gland with paroxysmal hypertension and concomitant swelling of the thyroid, 30\*
- tests in diagnosis of: cold pressor; histamine (Roth-Kvale); pyribenzamine and histamine; normal saline; calcium gluconate; nicotinic acid; epinephrine, 475\*
- with diabetes; 17-ketosteroid excretion in, 716\*

**PHOSPHATASE**, acid and alkaline, in carcinoma of the breast, 423\*

- acid, in seminal fluid, in sterility, 604

**PHOSPHORUS**: *see also* Parathyroids

- in serum, in carcinoma of the breast, 423\*
- metabolism, in hyperparathyroidism with myxedema, 152\*
- phospholipid phosphorus in blood in thyroid disease, 76

**PIGEON**, carbohydrate and fat metabolism in: cyclic glycemic and lipemias associated with egg production, 661**PIGMENTATION**: *see also* Addison's disease

- of skin: Addison's disease in Negro, 69
- of skin, in polyostotic fibrous dysplasia, 307\*
- of skin, marked, in Addison's disease; occurrence of vitiligo or leukoderma during therapy, 134\*
- melanoderma, as a sequel of head trauma, 303

**PITUITARY**: *see also* Cushing's syndrome; Gonadotropins**DISORDERS**

- *acromegaly*
  - amyotrophic type; treatment with testosterone propionate, 636\*
  - and amyotrophic lateral sclerosis, 636\*
  - and diabetes mellitus; disappearance of diabetes following acute mastoiditis and basilar meningitis, 455†
  - and diabetes mellitus; plasma protein pattern in, 559\*
  - estrone clearance test in, 519\*
  - plasma protein pattern in, 559\*
  - treatment with thiouracil; development of goiter and myxedema, 235\*

**PITUITARY** (cont.)**DISORDERS** (cont.)

- urinary glyco-genic corticoids in, 79\*
- urinary gonadotropins in, 1\*
- urinary 17-ketosteroids in, 79\*
- Cushing's syndrome: *see* Cushing's syndrome
- *diabetes insipidus*
  - compared with psychogenic polydipsia; use of hypertonic saline infusions in differential diagnosis of; effect of pitressin intravenously, 464†, 753\*
  - 17-ketosteroid excretion in, 753\*
  - treatment of, by intranasal use of posterior pituitary powder, 753\*, 828\*
  - with postpartum pituitary necrosis, 75
- *dwarfism*
  - hypophyseal; comparison with "rudimentary ovary" syndrome, 385\*, 665\* (*see also* Pituitary, disorders, syndrome)
  - pregnancy in; congenital abnormalities of fetus, 532
- gigantism, urinary gonadotropins in, 1\*
- hypo-adrenal function of pituitary origin; plasma protein pattern in, 559\*
- *hypopituitarism*
  - excretion of 11-oxycorticosteroids and 17-ketosteroids in, 331\*
  - hypopituitarism, hypo-adrenalism and diabetes mellitus (post-thyroidectomy), 376
  - possible role of, in depression of renal function in myxedema, 801\*
- hypothyroidism, pituitary type, with impaired renal function, 74
- irradiation of pituitary in treatment of hyperophthalmopathy syndrome in thyrotoxicosis, 102\*
- *panhypopituitarism*
  - associated with enlargement of the sella turcica, 79\*
  - following hemorrhage at the time of labor, 79\*
  - in patient followed 22 years after removal of craniopharyngioma, 79\*
  - 17-ketosteroid and glyco-genic corticoid excretion in, 79\*
- "Pan hypopituitary" eunuchoidism; spermatogenesis in, as a result of testosterone therapy, 781\*
- Simmonds' disease: effect of 11-DHCA on arginase activity of red blood cells in, 325\*



## PITUITARY (cont.)

## DISORDERS (cont.)

- syndrome of rudimentary ovaries, sexual infantilism, short stature, congenital defects
- increased urinary gonadotropins in, 11\*, 385\*, 609, 665\*
- absence of urinary gonadotropin in, 807\*
- *tumors*
  - adenomas, chromophobe, 466†
  - craniopharyngiomas, 466†
  - craniopharyngiomas in children; related lesions of hypophysis and hypothalamus; evaluation of treatment, 376
  - parapituitary tumors, 466†
  - studies on, 466†

FOLLICLE-STIMULATING HORMONE (F.S.H.);  
GONADOTROPINS: HORMONES: *see* Gonadotropins; and Pituitary, preparations

## PATHOLOGY AND PHYSIOLOGY

- anemia induced by hypophysectomy in rats; effect of iron, copper, and thyroxine on, 376
- as mediator in reduction of urinary glycogenic corticoids produced by testosterone therapy, 729\*
- changes in basophilic cells, following administration of antigonadotropic serum to rats, 447†
- changes in basophilic cells; in unusual case of Cushing's syndrome with spontaneous remission, followed by virilism, 787\*
- diffuse fibrosis of, in Addison's disease, 134\*
- function, in hyperophthalmopathic syndrome of thyrotoxicosis; suppression of, by administration of estrogens, 102\*
- gonadotropin content of, following administration of antigonadotropic serum to rats, 447†
- inhibitory effect of dienestrol on, 688\*
- necrosis of, acute postpartum, 531
- necrosis, postpartum, with diabetes insipidus, 75
- weight; effect of estrogen and thiouracil on, in rats, 531

## PREPARATIONS

- anterior, lyophilized suspension: adrenotropic, renotropic and cardiotropic activity of, in thyroidectomized rats, 303
- *adrenocorticotrophic hormone*
  - changes in circulating leukocytes induced by, in normal subjects

## PITUITARY (cont.)

## PREPARATIONS (cont.)

- and in Addison's disease, 458†
- effect on rat's adrenal cortex and thymus; chemical and cytochemical studies, 457†
- in normal human female urine; effect on ascorbic acid level in adrenal, 71
- metabolism of electrolytes, nitrogenous principles, steroids, etc., following administration to human subjects, 458†, 459†
- urinary uric acid-creatinine ratio following administration of, 459†
- *diabetogenic hormone*
  - diabetes produced by pituitary extracts, 304
  - diabetogenic extract; effect on growth and diabetes in normal animals, 75
- follicle-stimulating hormone (F.S.H.): *see* Gonadotropins
- *gonadotropic hormone*: *see also* Gonadotropins; Pregnancy; Tests
  - anterior pituitary reaction in pregnancy; determined by hyperemia AZT, 159\*
  - anterior pituitary reaction in pregnancy; determined by influence upon spermatogenic activity of testis, in toad, 653\*
  - gonadal stimulation following administration of antigonadotropic serum to rats, 447†
  - gonadotropin (equine); development of antihormones, 466†
- lactogenic hormone; effect of estrogen and thiouracil on, in rats, 531
- posterior pituitary extract; in treatment of prolonged labor, 532
- *thyrotropic hormone*
  - thyroid stimulating hormone in blood of rats treated with thiourea, 378
  - effect of iodine and adrenalin on, in Graves' disease, 532
  - effect on ability of thyroid to collect radioactive iodine, 235\*
  - effect on radio iodine uptake, in metastases of thyroid carcinoma, 467†
  - inhibition of action by administration of thyroxine, in rats, 235\*, 251
  - production of exophthalmos and changes in fat metabolism in guinea pig, 305

PLASMA: *see* Blood

**POLYDIPSIA**, psychogenic, compared with diabetes insipidus; use of hypertonic saline infusions in differential diagnosis of, 464†, 753\*

**POLYOSTOTIC FIBROUS DYSPLASIA**, a defense of the entity, 307\*

— testosterone therapy in; improvement of vision, 455†

**POLYURIA**: effect in hydrated subjects of infusion of hypertonic saline, in healthy young adults; in polyuria of both neurohypophyseal and psychogenic origin; in diabetes mellitus, with and without polyuria; and in renal insufficiency, calculi and calcinosis, 464†, 753\*

**POSTGRADUATE COURSE IN ENDOCRINOLOGY**: *see* Association

**PRECOCIOUS PUBERTY**: *see* Puberty, precocious

**PREGNANCY**: *see also* Estrogens; Fetus; Gonadotropins; Menstruation; Ovary; Sterility; Tests; Uterus

— *abortion*; pregnandiol precipitation test as aid in management of, 351\*

— *habitual*; preconceptual progestin therapy for, 609

— *blood gonadotropins and vaginal smears in*; correlation with sex of fetus, 450†

— *effect of thiouracil derivatives in*, 47\*

— *estrone clearance in*, 519\*

— *glucuronidase activity in tissues of endometrium, myometrium, placenta and ovary*, 535\*

— *labor*; induction with methergine, 606

— *prolonged*; treatment with posterior pituitary extract, 532

— *uterus*; relation of hydrostatic condition of, to size and shape of conceptus, 474

— *pregnanediol excretion, antepartum and postpartum*, 608

— *pregnanediol excretion increased by diethylstilbestrol*, 609

#### COMPLICATIONS AND SEQUELAE

— *and diabetes insipidus*; postpartum pituitary necrosis, 75

— *and diabetes mellitus*; effect of diabetic and prediabetic pregnancies on fetus and newborn infants, 727, 728

— *maternal, obstetrical, chemical, fetal and placental abnormalities*, 471

— *treatment with insulin*; effect on maternal and infant mortality, 230

— *and hyperthyroidism*; congenital abnormality in fetus, 767\*

#### PREGNANCY (cont.)

##### COMPLICATIONS AND SEQUELAE (cont.)

— *treatment with thiouracil*; effect on fetus and on fetal thyroid, 47\*, 469†, 767\*

— *and infectious hepatitis*; estrone clearance in, 519\*

— *Chiari-Frommel syndrome*; historical review and case report, 608

— *hemorrhage at time of labor*: followed by acute postpartum necrosis of pituitary, 75, 531

— *followed by panhypopituitarism*; excretion of glycogenic corticoids and 17-ketosteroids in, 79\*

— *hydatidiform mole*; relation to eclampsia, 605

— *chorionic tumor*; use of South African frog for diagnosis of, 289\*

— *in pituitary dwarfism*; congenital abnormalities of fetus, 532

— *late pregnancy accidents*; prevention of, by diethylstilbestrol therapy, 609

— *necrosis of pituitary, acute, post partum*, 75, 531

— *lozemas of*: glucuronidase activity in serum during normal and toxemic pregnancy; effect of stilbestrol on, in normal puerperium, 535\*

— *thromboplastin*; relation to toxemia of pregnancy; antithromboplastin, 473

#### DIAGNOSIS

— *comparison of color chemical test for pregnanediol, with Friedman test*, 608

— *determination of time of conception*, 606

— *estimation, colorimetric, of urinary pregnanediol*, 457†

— *Guterman test, evaluation of*, 608

— *Hogben test, evaluation of*, 606

— *hyperemia AZ test*, 159\*

— *rapid pregnandiol precipitation test for diagnosis of*, 351\*

— *rapid test for, using male toad*, 653\*

**ESTROGENS (PROGESTERONE) IN**: *see* Estrogens

**GONADOTROPINS IN**: *see* Gonadotropins

**URINE IN**: *see* Estrogens; Gonadotropins; Pregnancy, diagnosis

**PREGNANE DERIVATIVE**, urinary, in precocious puberty, 171\*

**PREGNANEDIOL** (pregnane-diols and -triols): *see also* Estrogens, in urine; Pregnancy, diagnosis

— *comparison of color chemical test for, with Friedman test*, 608

## PREGNANEDIOL (cont.)

- *excretion*; antepartum and postpartum, 608
- in arrhenoblastoma, 607
- increased by diethylstilbestrol, 609
- in menstrual cycle and in study of corpus luteum function, 446†
- in precocious puberty, 171\*
- precipitation test for diagnosis of pregnancy, 351\*

PREGNANELONE: *see* EstrogensPREGNENOLONE: *see* EstrogensPREMATURE INFANTS: *see* Infants

PREPUCE: developmental separation from glans penis by topical application of steroid hormones, in human infants and in squirrels, 192\*

PROGESTERONE: *see* Estrogens, effects, therapy, etc.; Menstruation; Steroids; UterusPROGRAMS: *see* Association

PROMIZOLE: goitrogenic activity of, 378

PROPYLTHIOURACIL: *see* ThiouracilPROSTATE: *see* incidental to disorders of Testis; effects of Androgens, and Gonadotropins

## PROTEIN METABOLISM

- influence of protein intake on excretion of 11-oxycorticosteroids, 331\*
- plasma protein pattern (determined by Tiselius electrophoretic technique) in various endocrinopathies; effect of treatment, 559\*
- catabolism of; role of adrenal cortex in, following trauma, 461†

PSEUDOHERMAPHRODISM: *see* Hermaphrodisism

PSYCHIC FACTORS: behavior and psychologic problems of young diabetic patients, 231

- differential diagnosis of diabetes insipidus and psychogenic polydipsia; use of hypertonic saline infusions in, 753\*
- emotional factors in obesity, 225
- estrogenic hormones, as psychic factor, in treatment of menopause, 228
- psychiatric and endocrinological factors in the menopause, 226
- psychoneurosis associated with hyperostosis frontalis interna, etc., 624\*
- relation to menstrual misbehaviors, 659\*
- social and psychological readjustment under endocrine therapy, in pseudohermaphrodisism, 456†

PSYCHOSIS, manic-depressive, in hyperparathyroidism, following removal of parathyroid adenoma; relationship of alterations in calcium and phosphorus metabolism to, 152\*

PUBERTY: *see also* Children; Growth; Menstruation; Ovary; Testis; Virilism  
— in girls with diabetes mellitus, in relationship to level of control of disease, 727

## PRECOCIOUS

- associated with polyostotic fibrous dysplasia, 307\*
- constitutional type; comparison with other types; sugar tolerance; excretion of gonadotropins and steroids; effect of progesterone and of unilateral ovariectomy in, 171\*
- differential diagnosis; ovarian pathology; excretion of gonadotropin and 17-ketosteroids; effect of removal of polycystic ovary, 11\*
- following administration of anti-gonadotropic serum, in rat, 447†
- "macrogenitosomia"; congenital adrenocortical insufficiency associated with, 604
- urinary steroids in, 543\*

RADIOACTIVE IODINE: *see* IodineRADIO IODINE: *see* IodineREPRODUCTION: *see* Menstruation; Pregnancy; Ovary; Sterility; Testis

RESPIRATORY QUOTIENT: in diabetes mellitus following oral ingestion of glucose and fructose, with and without insulin, 472

ROENTGEN RAY THERAPY: *see* Irradiation

ROTH-KVALE (HISTAMINE) TEST in diagnosis of pheochromocytoma, 475\*

RUDIMENTARY OVARY SYNDROME: *see also* Ovary; Syndrome

- anatomical, biological and clinical manifestations, 388\*

SALIVARY GLANDS, swelling of, with salivation, during sublingual application of desoxycorticosterone acetate in treatment of Addison's disease, 134\*

SARCOID, hypercalcemia and corneal lesions in, 644\*

SCHERING award and research funds; *see* Association, newsSEMEN: *see* Testis, spermatozoaSERUM: *see* BloodSEX: *see also* Gonad; Hermaphrodisism; Ovary; Testis

- characteristics, secondary: (*see also* Breast; Hair)
  - auricular hairs; relation to race, endocrine stimulation and urinary steroids, 465†
  - distribution in arthritis, 201\*

## SEX (cont.)

- hormones: *see* Androgens; Estrogens; Steroids
- of fetus; correlation of blood gonadotropins and vaginal smears of mother with, 450†

SIMMONDS' DISEASE: *see* Pituitary, disorders

SKIN, lupus erythematosus disseminatus; effect of testosterone on excretion of glyco-genic corticoids and of 17-ketosteroids in, 729\*

- pigmentation: *see* Addison's disease; and Pigmentation
- striae: *see* Cushing's syndrome
- telangiectasis in thyrotoxicosis; relation of amount of axillary hair to, 52\*

SLEEP, effect on excretion of 17-ketosteroids in urine, 264\*

SMEAR: *see also* Menstruation; Ovary

- urethral; evaluation of, as index of androgenic deficiency in the male, 186\*
- vaginal and cervical; in comparative study of cornification in human subjects in various stages of menstrual cycle, 749\*
- vaginal; in menopause; effect of estrogen therapy, 451†
- vaginal; in pregnancy; correlation with sex of fetus, 450†
- vaginal, in "rudimentary ovary" syndrome, 385\*
- vaginal, in study of clinical effects of dienestrol, 688\*

SOCIAL and psychological readjustment under endocrine therapy in pseudohermaphroditism, 456†

- and psychologic problems of young diabetic patients, 231

SODIUM: *see also* Addison's disease; and Chloride

- method for estimation of, in body fluids, 72

SPERMATOZOA: *see* Testis

SQUIBB, E. R. AND SONS award: *see* Association, awards

SQUIRREL, effect of topical application of steroid hormones on separation of prepuce from glans penis; on prostate; and on seminal vesicles in, 192\*

STERILITY: *see also* Eunuchoidism; Hypogonadism; Menstruation; Ovary, Pregnancy; Testis, spermatozoa

- azoospermia without impairment of Sertoli or Leydig cells of testis; gonadotropic and 17-ketosteroid excretion in, 493\*
- in female; analysis of 257 cases, 610
- oligospermia in male gynecomastia, 586\*

## STERILITY (cont.)

- semen analysis in, 605
- seminal fluid acid phosphatase in, 604
- spermatogenesis in "pan-hypopituitary" eunuchoid, as a result of testosterone therapy, 781\*

STEROIDS: *see also* Adrenals, steroids; Androgens; Estrogens; Urine

ANDROGENS: *see* Androgens

CORTICOSTEROIDS, CORTICOIDS: *see* Adrenals, steroids

ESTROGENS: *see* Estrogens

## 17-KETOSTEROIDS

- in urine, decreased excretion caused by estrogen therapy in normal male, 186\*

- during progesterone therapy of uterine fibromyomata, 446†

- effect of administration of adrenocorticotrophic hormone of pituitary to human subjects, 458†, 459†

- effect of chronic debility on, 264\*

- effect of dienestrol therapy on, 688\*

- effect of sleep on, 264\*

- effect of testosterone on, 729\*

- effect of trauma and disease on, 264\*

- in a case of ovotestis; effect of removal of ovotestis on, 455†, 741\*

- in acromegaly, 636\*

- in ankylosing spondylarthritis and in rheumatoid arthritis, 201\*

- in anorexia nervosa, 79\*

- in arrhenoblastoma, 607

- in azoospermatic testicular syndrome, 493\*

- in burns, 331\*

- in cretinism, 503\*

- in Cushing's syndrome, 543\*, 559\*

- in endocrine and other disorders, 79\*

- in eunuchoidism, 130\*

- in eunuchoidism with hyperthyroidism, 566\*

- in hermaphroditism, 543\*

- in hypogonadal male, 186\*

- in male gynecomastia, 586\*

- in mongolism, 503\*

- in myotonia dystrophica, 503\*

- in normal and abnormal subjects; effect of treatment on, 331\*

- in oligophrenia (defective mental development), 503\*

- in "pan-hypopituitary" eunuchoidism, 781\*

## STEROIDS (cont.)

## 17-KETOSTEROIDS (cont.)

- in pheochromocytoma with diabetes, 716\*
- in precocious puberty, 11\*, 171\*, 543\*
- in syndrome of rudimentary ovaries, short stature, sexual infantilism, etc., 11\*, 385\*, 665\*, 807\*
- in virilizing ovarian tumor; effect of removal of tumor, 115\*
- in virilism, 11\*, 331\*
- level, correlated with histology of adrenal, 503\*
- methods for determination of, 451†, 453†, 795

11-OXYCORTICOSTEROIDS: *see* Adrenals, steroids

STEROIDS, and adrenal extracts; assay for protective action against toxins, 69

- color reactions of, 364\*, 452†, 453†, 454†, 457†, 701\*
- color reactions of: meta-dinitrobenzene and antimony-trichloride, 454†
- effects of topical application of, on developmental separation of the prepuce from the glans penis, 192\*
- estrogenic; simple quantitative colorimetric method for estimation of, 701\*
- *in urine*:
  - in adrenal virilism and in pseudohermaphroditism; effect of adrenalectomy on, 533
  - in normal Caucasian men; relation to auricular hair, 465†
  - in obesity, 462†
  - photometric estimation of estrogens in, 364\*
  - relation to diagnosis of adrenal cortical tumors and adrenal cortical hyperplasia; quantitative and isolation studies, 543\*
  - utilization of liquid chromatogram technique in colorimetric estimation of pregnanediol, 457†
  - various types of steroid hormones; measurement of, by concomitant application of Zimmermann and Kober reactions, 453†
- sex, relationship to weight of adrenal glands in hamsters and rats, 463†

STILBESTROL: *see* Estrogens

STREPTOMYCIN in the treatment of thiouracil agranulocytosis, 219\*

SUGAR: *see* Carbohydrate metabolism: Diabetes mellitus

SYNDROME, Chiari-Frommel; historical review and case report, 608

- Cushing's: *see* Cushing's syndrome
- hyperophthalmopathic, in thyroid disease, 102\*
- hyperostosis frontalis interna, not a clinical entity, 624\*
- Laurence-Moon-Biedl; description of two cases, familial, 780
- rudimentary ovaries, short stature, sexual infantilism (*see* Ovary), 11\*, 385\*, 665\*, 807\*
- testicular; produced by absence of germinal epithelium without impairment of Sertoli or Leydig cells; gonadotropic and 17-ketosteroid excretion in, 493\*

TABLES, for predicting height of children, 473

TEMPERATURE body, correlation of, with endometrial biopsy, 451†

- body; correlation of, with viscosity of cervical mucus and ovulation, 610
- effect on speed of pregnancy test, 653\*
- effect on hydrolysis in determination of total urinary 17-ketosteroids, 795\*
- skin and rectal, in men, during extreme cold, 73

TESTIS: *see also* Androgens; Gonad; Gonadotropins; Hypogonadism; Sterility

- absence of germinal epithelium without impairment of Sertoli or Leydig cells; urinary gonadotropic and 17-ketosteroid excretion in, 493\*
- effect of castration on blood regeneration in thyroidectomized rats, 306
- hermaphroditism: (*see also* Hermaphroditism) a case of ovotestis, 741\*
- new testicular hormone; specific function of Sertoli cells established, 493\*
- undescended, bilateral, 566\*

PREPARATIONS: *see* Androgens

SPERMATOZOA, acid phosphatase in seminal fluid in sterility, 604

- azoospermia, without impairment of endocrine function, 493\*
- in sterility; semen analysis, 604, 605
- oligospermia associated with gynecomastia, 586\*
- seminiferous tubule failure; urinary gonadotropins in, 1\*
- spermatogenesis; effect of pregnenolone and of testosterone on, 227
- spermatogenesis in a "pan-hypopituitary" eunuchoid, as the result of testosterone therapy, 781\*
- spermatogenic activity in toad, as influenced by chorionic gonadotropin, 653\*

## TESTIS (cont.)

- TUMORS, adenoma testiculare of Pick, without masculinization; large breasts; high urinary gonadotropins, 456†
- androblastoma, 228
- feminizing testicular tumor with atrophy of other testis, gynecomastia, increased excretion of estrogen and absence of gonadotropin in urine, effect of removal, 438\*
- homologous ovarian and testicular, 227, 228
- gonocytoma, 227

TESTOSTERONE: *see* AndrogensTESTOSTERONE PROPIONATE: *see* AndrogensTESTS: *see also* Methods

- antimony-trichloride, in color reactions of steroids, 454†
- Aschheim-Zondek, new hyperemia AZT, 159\* (*see also* Gonadotropins; Pregnancy, diagnosis; Tests, for pregnancy)
- basal metabolism; method for assuring accuracy in, 304
- color reactions of steroids, 364\*, 452†, 453†, 454†, 457†, 701\*
- estrone clearance test for liver disease, 519\*
- for adrenal cortical function; urinary uric acid-creatinine ratio, following administration of ACTH, 459†
- for estrogens; quantitative colorimetric, 364\*, 452†, 701\*
- for hydatidiform mole; gonadotropic hormone assay in urine, using South African frog, 289\*
- for pregnancy
  - colorimetric estimation of urinary pregnanediol, 457†
  - comparison of color chemical test with Friedman modification of the Aschheim-Zondek test, 608
  - for determining the time of ovulation and conception, 606
  - Guterman, evaluation of, 608
  - Hogben, evaluation of, 606
  - hyperemia AZ test, 159\*
  - rapid pregnanediol precipitation test, 351\*
  - rapid, using male toad, 653\*
- in diabetes mellitus
  - Exton-Rose (two-dose) sugar tolerance test, in diagnosis, 229
  - fallacy of, 232
  - for ketosis, 229, 230
  - simple, for blood ketones in diabetic acidosis, 230
- Kober reaction; modification of, for estimation of estrogenic steroids, 701\*

## TESTS (cont.)

- Kober reaction, used concomitantly with Zimmermann reaction for estimation of steroid hormones in urine, 453†
- meta-dinitrobenzene, in color reactions of steroids, 454†
- Roth-Kvale (histamine) in diagnosis of pheochromocytoma, 475\* (*see* Pheochromocytoma)
- urethral smear test for androgenic deficiency, 186\*
- use of hypertonic saline infusions in differential diagnosis of diabetes insipidus and psychogenic polydipsia, 464†, 753\*
- Zimmermann and Kober reactions (*see* Tests, Kober)

TETANY: *see* Parathyroids

THIOCYANATE, potassium; effect on collection of radioactive iodine by thyroid, 235\*

- production of goiter and of myxedema by, 235\*

## THIOURACIL

## EFFECTS

- and estrogen; effect of, on lactogenic hormone and weight of pituitary, 531
- derivatives of thiouracil:
  - effect of transmammary administration of, in rat, 47\*
  - effect on fetuses and infants, 47\*, 381
  - thyroid hormone administered with; prevention of thyroid hyperplasia in rat, 47\*
  - transplacental transmission of, in rat, 47\*
- effect on collection of radioactive iodine by thyroid, 235\*
- effect on creatine metabolism in thyrotoxicosis, 381
- effect on fetus, 47\*, 381, 469†
- effect on iodine content of human fetal thyroid, 767\*
- effect on mitotic activity and wound healing in corneal epithelium of rat, 468†
- effect on radioiodine uptake in metastases of thyroid carcinoma, 467†
- inhibitory effect on blood regeneration in rat, 306
- production of goiter and of myxedema by, 235\*

## THERAPY

- combined with radioiodine, in toxic goiter, 235\*
- in diabetes mellitus associated with hyperthyroidism, 234

## THIOURACIL (cont.)

## THERAPY (cont.)

- in hyperthyroidism, 76, 77, 78, 102\*, 219\*, 234, 235\*, 305, 377, 379, 380, 381, 566\*, 767\*, 812\*, 830\*
- in hyperthyroidism complicating pregnancy, and its effect on the human fetal thyroid, 767\*
- in hyperthyroidism without goiter, 377
- in leukemia, 225, 780
- in thyrotoxicosis, following treatment with aminothiazole, 812\*
- methylthiouracil in treatment of hyperthyroidism in pregnancy; effect on thyroid gland of fetus, 47\*
- observations on the use of, in thyroid disease, 380
- propylthiouracil in preoperative preparation of thyrotoxic patients, 212\*
- propylthiouracil in treatment of hyperthyroidism, 304, 377
- 2-thiouracil, in treatment of congestive heart failure, 74

## TOXICITY

- agranulocytosis from, in treatment of hyperophthalmopathic syndrome in thyroid disease, 102\*
- agranulocytosis from; streptomycin therapy in, 219\*
- cause of perosis in chicks, 76
- complications following administration of; prevention of agranulocytosis, 305
- fatal agranulocytosis from, in treatment of thyrotoxicosis, 78
- fatal jaundice from, 830\*
- granulopenia during treatment of hyperthyroidism associated with eunuchoidism, 566\*

THIOUREA; thyroid stimulating hormone in blood of rats treated with, 378

THROMBOPLASTIN; active principle of placental toxin; antithromboplastin, 473

THYMUS, aberrant parathyroid tissue located in, 152\*

- effect of pituitary adrenocorticotrophic hormone on, in rat, 457†

THYROID: *see also* Iodine; Thiouracil

## DISORDERS

- adenomas; clinical significance of functional behavior of, 380
- adenomas; radio iodine fractionation studies of, 380
- carcinoma, metastatic, studied with radioactive iodine, 467†
- correlation between structure and function of benign and malignant tumors, studied by radioactive iodine, 235\*

## THYROID (cont.)

## DISORDERS (cont.)

- neoplasia; possible relation to hyperplasia, 77
- thyrocardiac disease, value of thyroidectomy in, 379
- GOITER, endemic goiter and iodine-lack theory; prophylaxis, 58\*, 60\*, 62\*, 820\*
- induced by promizole, in animals; effect of graded doses of thyroxine on, 378
- induced by therapy with potassium thiocyanate and with thiouracil; excretion of radio iodine in, 235\*
- in two brothers with sporadic cretinism, 77
- on an iodine-free diet grown by hydroponics, and excluding goiter noxa, 714\*
- paroxysmal swelling of thyroid in pheochromocytoma, 475\*
- surgery of the thyroid gland, 379
- *toxic* (exophthalmic goiter; Graves' disease) *see also* Thyroid, hyperthyroidism
  - changes in prominence of eyes in various thyroid states, 306
  - hyperophthalmopathic syndrome; treatment; urinary 17-ketosteroids, estrogens and gonadotropins in, 102\*
  - intractable exophthalmos, surgery of, 379
  - pathogenesis of cirrhosis of liver in, 379
  - radio iodine; excretion in urine and collection by thyroid, 235\*
  - some vulgar errors regarding diagnosis; plea for early surgery, 77
  - therapy: (medical and surgical) *see* Hyperthyroidism under Thyroid; Iodine; Thiouracil
  - thyrotropic hormone of pituitary; effect of iodine and adrenalin on, in Graves' disease, 532

## HYPERTHYROIDISM

- antagonism to, and treatment with vitamin A; effect on blood cholesterol, 574\*
- cholesterol, cholesterol esters and phospholipid phosphorus in blood, 76
- creatine metabolism in, 381
- metabolism of radio iodine in, 235\*
- specific gravity and protein content of blood plasma, 75
- specific renal functions in, 801\*
- without goiter, 377

## THYROID (cont.)

## HYPERTHYROIDISM (cont.)

- *complications and sequelae*, associated with diabetes mellitus, amenorrhea, and melanoderma; recurrence caused by head trauma, 303
- associated with diabetes mellitus in two combinations; effect of treatment with thiouracil, 234
- complicating pregnancy; treatment with thiouracil; effect on human fetal thyroid, 47\*, 469†, 767\*
- hypotrichosis axillaris in thyrotoxicosis; correlation with telangiectasis of skin, "liver palms," and gynecomastia, 52\*
- in a prepueral eunuchoid; thiouracil therapy, 566\*
- iodism, severe, as a complication, 212\*
- parathyroid insufficiency, following thyroidectomy, 433\*
- *therapy*, agranulocytosis from thiouracil therapy (*see* Thiouracil), 78, 102\*, 219\*, 305, 566\*
- estrogenic therapy in, 380
- fatal agranulocytosis from thiouracil therapy, 78
- fatal jaundice from thiouracil therapy, 830\*
- radiation therapy in, 380
- surgery of thyroid gland, 379
- with aminothiazole, 812\*
- with iodine: *see* Iodine
- with radio iodine, 78, 235\*, 377, 378, *see also* Iodine, radio
- with streptomycin; in agranulocytosis caused by thiouracil, 219\*
- with thiouracil and derivatives, 76, 77, 78, 102\*, 219\*, 234, 235\*, 305, 377, 379, 380, 381, 566\*, 767\*, 812\*, 830\* (*see* Thiouracil)

## HYPOTHYROIDISM

- cholesterol, cholesterol esters and phospholipid phosphorus in blood, 76
- effect on creatine metabolism, 381
- excretion of 11-oxy corticosteroids and of 17-ketosteroids in, 331\*
- pituitary type, with impaired renal function, 74
- *cretinism*, and iodine-lack theory, 820\*
- 17-ketosteroids in urine, 503\*
- sporadic, in two brothers with goiter, 77
- *myxedema*, and heart disease; differential diagnosis, 468†

## THYROID (cont.)

## HYPOTHYROIDISM (cont.)

- controlled by thyroid extract for 52 years, 76
  - menstruation in, 467†
  - produced by methylthiouracil, in rat: prevention of, by administering thyroid hormone, 47\*
  - produced by thiouracil and hypokassium thioeyanate, 235\*
  - produced by 2-thiouracil, 74
  - specific renal functions in, 801\*
  - urinary gonadotropins in, 1\*
  - with hyperparathyroidism; thyroid therapy in, 152\*
  - *therapy*: *see* Thyroid, preparations
- PHYSIOLOGY AND PATHOLOGY
- and adrenal interrelations, 52\*
  - carbohydrate appetite of hyperthyroid rats, determined by taste-threshold method, 467†
  - effect of liver feeding on growth and ovarian development in hyperthyroid rats, 532
  - effect of thiouracil therapy in mother on iodine content of fetal thyroid, 47\*, 469†, 767\*
  - effect of thiouracil therapy in mother rats and rabbits on thyroids of suckling offspring, 47\*
  - inhibitors; effect of, on the metabolism of iodine and on formation of thyroxine, 235\*
  - nerve supply; role in autotransplantation of, 75
  - physiology; use of radio iodine in the study of, 235\*
  - thiouracil and potassium thioeyanate; effect on collection of radioactive iodine by thyroid gland, 235\*
  - thyroid stimulating hormone in blood of rats treated with thiourea, 378
  - thyrotropic hormone of pituitary; effect on ability of thyroid to collect radioactive iodine, 235\*
  - thyrotropic hormone of the pituitary, producing exophthalmos and changes in fat metabolism, in guinea pig, 305
- PREPARATIONS
- diiodotrypsine; steps in synthesis of, as studied by transformation of radioactive iodine, 235\*
  - effect of treatment with, in case of congenital defects, short stature, retarded sexual development, no urinary gonadotropins, 807\*
  - effect on menstruation, 467†



## THYROID (cont.)

## PREPARATIONS (cont.)

- thyroid extract; in control of myxedema in one patient for 52 years, 76
- thyroid hormone, administered with methylthiouracil; prevention of thyroid hyperplasia in rat, 47\*
- thyroxine; and thyroid extract, in treatment of hyperophthalmopathic syndrome in thyroid disease, 102\*
- thyroxine; effect of graded doses on experimental goiter induced by promizole, 378
- thyroxine; effect of, on response of hypophysectomized rat's thyroid to thyrotropic hormone, 251
- thyroxine; synthesis of, as studied by transformation of radioactive iodine, 235\*
- thyroxine, with and without cobalt; stimulating effect on blood regeneration following thyroidectomy, in rat, 306

THYROTOXICOSIS: *see* Thyroid, goiter and hyperthyroidism

THYROXINE: *see* Thyroid, preparations

TIME sensitivity to chorionic gonadotropin in hyperemia AZ test, 159\*

TISELIUS electrophoretic technique in determination of plasma protein pattern, 559\*

TOAD, male, used for rapid pregnancy test, 653\*

TOXIC ADENOMA: *see* Thyroid, hyperthyroidism

TOXIC GOITER: *see* Thyroid, goiter and hyperthyroidism

TOXINS, bacterial; protective power of adrenal preparations against, 460†

TRANSPLANTATION: intraocular transplants of endometrium, in study of menstrual cycle in monkey, 449†, 611\*

TRAUMA: effect on urinary cortin, 70, 70

— effect on urinary 17-ketosteroids, 264\*

— of head, causing reactivation of thyrotoxicosis with associated diabetes, amenorrhea and melanoderma, 303

— subsequent protein catabolism; role of adrenal cortex in, 461†

TUBERCULOSIS: *see also* incidental to Addison's disease

— lupus erythematosus disseminatus; effect of testosterone on excretion of glycoenic corticoids and of 17-ketosteroids in, 729\*

## TUMORS:

— adenoma testicular of Pick, *see* Ovary and Testis, tumors

## TUMORS (cont.)

- arrhenoblastoma, *see* Ovary, tumors
  - chromaffinoma, *see* Pheochromocytoma
  - chromophobe adenoma, *see* Pituitary
  - clinical significance of functional behavior of adenomas; radio iodine fractionation studies, 380
  - correlation between structure and function of benign and malignant tumors; radio iodine studies, 235\*
  - craniopharyngioma, *see* Craniopharyngioma; and Pituitary
  - endothelioma, treatment, *see* Estrogen, therapy
  - feminizing, of testis, *see* Testis, tumors
  - fibromyomata, *see* Uterus
  - gonocytoma, *see* Ovary and Testis, tumors
  - granulosa-cell, *see* Ovary, tumors
  - homologous ovarian and testicular, *see* Ovary and Testis, tumors
  - hydatidiform mole, *see* Hydatidiform mole; and incidental to Pregnancy, tests
  - neoplasia; possible relation to hyperplasia, 77
  - of adrenal cortex, *see* Adrenals, tumors
  - of adrenal medulla, *see* Pheochromocytoma
  - of breast, *see* Breast
  - of Ovary, *see* Ovary, tumors
  - of parathyroids, *see* Parathyroids
  - of pituitary, *see* Pituitary, tumors
  - of testis, *see* Testis, tumors
  - of thyroid, *see* Thyroid
  - mammary, *see* Breast
  - pheochromocytoma, *see* Pheochromocytoma
  - therapy, *see* Androgens; Estrogens; Iodine, radioactive
  - virilizing, *see* Ovary, tumors
- TWINS, in observations on the effect of testosterone in premature infants, 708\*
- TYPHOID VACCINE, in assay of protective power of adrenal extracts and steroids, 69

ULCER, duodenal, peptic; *see* Gastrointestinal tract

UNIT; hyperemia rat unit of chorionic gonadotropin, evaluation of, 159\*

URETHRA: urethral smear; evaluation of, as index of androgenic deficiency in the male, 186\*

URINE: *see also* under various endocrine disorders (urine in); Androgens; Estrogens; Gonadotropins; Methods; Steroids; Tests

## URINE (cont.)

- adrenal corticosteroids in, *see* Adrenals, steroids
- androgens in, *see* Androgens
- calcium excretion in myxedema with hyperparathyroidism, 152\*
- chemistry: methods, *see* Methods; Tests
- chloride excretion during glycosuria, in diabetes, 231
- chorionic gonadotropin in, *see* Gonadotropins; Pregnancy, diagnosis; Tests
- corticosteroids (corticoids, cortin, etc.) in, *see* Adrenals, steroids
- estrogens in, *see* Estrogens
- estrone clearance, *see* Estrogens
- *flow*: effect in hydrated subjects, of infusion of hypertonic saline in:
  - diabetes mellitus, with and without polyuria, 753\*
  - healthy young adults, 753\*
  - polyuria of neurohypophyseal, and of psychogenic origin, 753\*
  - renal calcinosis, calculi, and insufficiency, 753\*
- excretion of electrolytes, nitrogenous principles, steroids, etc. following administration of adrenocorticotrophic hormone of pituitary to human subjects, 458†, 459†
- glycogenic corticoids in, *see* Adrenals, steroids
- gonadotropins in, *see also* Gonadotropins
  - in syndrome of rudimentary ovaries, short stature, sexual infantilism, etc.
    - absent, 807\*
    - increased, 11\*, 385\*, 609, 665\*
- 17-ketosteroids in, *see* Steroids
- melituria in healthy American men with reference to transitory glycosuria, 473
- methods of quantitative analysis, *see* Methods; Tests
- 11-oxy corticosteroids in, *see* Adrenals, steroids
- pituitary hormones in, other than gonadotropins (c.g., adrenocorticotrophic substance), *see* Pituitary, preparations
- pregnancy tests, *see* Pregnancy, diagnosis; Tests
- pregnanediol in, *see* Estrogens; Pregnancy
- radioactive iodine in, *see* Iodine, radioactive
- steroids in, *see* Adrenals; Androgens; Estrogens; Steroids

## URINE (cont.)

- tests for various constituents of, *see* Methods; Tests
  - uric acid-creatinine ratio, following administration of ACTH, as simple test for adrenal cortex function, 459†
- UTERUS: *see also* Estrogens; Menstruation; Pregnancy; Ovary
- abortion, habitual; preconceptional progestin therapy for, 609
  - abortion; pregnandiol precipitation test as aid in management of, 351\*
  - cervical mucus; cyclic changes in viscosity and amount; correlation with body temperature and ovulation, 610
  - comparison of cervical and vaginal cornification in human subjects in various stages of menstrual cycle, 749\*
  - endometrial biopsy,
    - correlation of basal body temperature curves with, 451†
    - demonstrating effect of stilbestrol on human ovarian cycle, 450†
    - in study of effect of orally administered estrogen, in the menopause, 451†
    - pregnanediol excretion correlated with; effect of chorionic gonadotropin on, 446†
  - endometrium: architecture of endometrial vascular bed in the monkey; influence of ovarian hormones on, 449†, 611\*
  - endometrium: intraocular transplants in study of structure of vascular bed in menstrual cycle in the monkey: effect of previous treatment on, 449†, 611\*
  - fibromyomata; progesterone therapy of, by intramuscular pellet implantation, 446†
  - glucuronidase activity in tissues of endometrium, myometrium, placenta and ovary, 535\*
  - hydatidiform mole; relation to eclampsia, 605
  - hydatidiform mole; diagnosis, using South African frog for assay of chorionic gonadotropin, 289\*
  - marked ovarian and uterine stimulation following administration of anti-gonadotropic serum, in rat, 447†
  - labor; induction with methergine, 606
  - labor, prolonged; pituitary extract therapy, 532
  - relation of hydrostatic condition of, to size and shape of conceptus, 474

VAGINA: *see also* Estrogens; Menstruation; Ovary; Uterus

## VAGINA (cont.)

- atrophic vaginitis; dienestrol therapy, 688\*
- comparison of vaginal and cervical cornification in human subjects in various stages of menstrual cycle, 749\*
- mucosa; effect of estrogenic therapy on hydrogen ion concentration of, 226
- smear; in clinical evaluation of dienestrol therapy, 688\*
- smear; in menopause; effect of estrogen therapy, 451†
- smear; in pregnancy; correlation with sex of fetus, 450†
- smear; in "rudimentary ovary" syndrome, 385\*

VAN METER PRIZE AWARD of American Association for the Study of Goiter, *see* Association, news

VASCULAR SYSTEM: *see also* Hypertension

- abnormalities of, in syndrome of congenitally aplastic ovaries, sexual infantilism, short stature, etc., 665\*
- artery, spiral, of ovary; effect of gonadotropins on, in rabbits, 447†
- endometrial, in monkey; study of, 449†
- hepato-renal factors in circulatory homeostasis; relation of adrenals to formation of a renal vaso-excitor principle, 460†
- renal blood flow, vascular resistance, etc. in hyperthyroidism and in myxedema, 801\*

VIRILISM: *see also* Adrenals; Cushing's syndrome; Hermaphroditism; Ovary; Puberty, precocious

- adrenal, associated with mental disorder; effect of adrenalectomy in, 533
- associated with adrenal-like ovarian tumor; 17-ketosteroids in urine; results of treatment, 115\*
- associated with ovarian hypertecosis; 17-ketosteroids and gonadotropins in urine; glucose tolerance; effect of ovarian wedge resection, 11\*
- following Cushing's syndrome; endocrine disorders associated with; unusual case, 787\*
- in a case of arrhenoblastoma; effect of removal of tumor, 606
- produced by testosterone propionate

## VIRILISM (cont.)

- in woman treated for carcinoma of breast, 423\*
  - urinary 11-oxycorticosteroids and 17-ketosteroids in, 331\*
  - urinary steroids in, 543\*, *see also* Steroids
- VITAMINS, ascorbic acid in plasma and urine during menstrual cycle, 607
- ascorbic acid level in adrenal; effect of adrenocorticotrophic substance in female human urine on, 71
  - vitamin A; antagonism to thyroid; use of massive doses in treatment of hyperthyroidism, and of pre-menstrual tension, 574\*
  - vitamin A, in relation to carotene metabolism in the diabetic, 232
  - vitamin C in the treatment of Addison's disease, 134\*
  - vitamin D poisoning; hypercalcemia; conjunctival and corneal lesions in, 464†, 644\*

VITILIGO: *see* Pigmentation

WATER METABOLISM: *see also* Pituitary, *diabetes insipidus*

- edema during estrogen therapy for dysmenorrhea, 450†
- hypertonic saline infusions in hydrated subjects, in the differential diagnosis of diabetes insipidus and psychogenic polydipsia, 464†, 753\*

WEIGHT: *see also* under effects of various endocrine disorders and preparations; Anorexia nervosa; Diet; Growth

- effect of diabetogenic extract of pituitary on, in normal animals, 75
- effect of testosterone propionate therapy on, in carcinoma of the breast, 423\*
- increase during estrogen therapy for dysmenorrhea, due to edema, 450†
- in premature infants; effect of testosterone compounds on, 465†, 708\*
- loss of, in men in internment camp, 71

XENOPUS LAEVIS (South African frog); use of, in diagnosis of hydatidiform mole, 289\*

X-RAY THERAPY: *see* Irradiation

ZIMMERMANN REACTION: *see* Tests

